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VARIATION IN SPECIES INTERACTIONS AND THEIR EVOLUTIONARY CONSEQUENCES

by

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ABSTRACT

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Species interactions restrict or promote population growth, structure communities, and contribute to evolution of diverse taxa. I seek to understand how multiple species interactions are maintained, how human altered species interactions influence evolution, and explore factors that contribute to variation in species interactions. In Chapter 1, I examine how plants interact with multiple guilds of mutualists, many of which are costly interactions. The evolution of traits used to attract different mutualist guilds may be constrained due to ecological or genetic mechanisms. I asked if two sets of plant traits that mediate interactions with two guilds of mutualists, pollinators and ant bodyguards, were positively or negatively correlated across 36 species of *Gossypium* (cotton). Traits to attract pollinators were positively correlated with traits to attract ant bodyguards. Rather than interaction with one mutualist guild limiting interactions with another mutualist guild, traits have evolved to increase attraction of multiple mutualist guilds simultaneously. In Chapters 2 and 3, motivated by the fact that agriculture covers nearly 50% of the global vegetated land surface, I explore the consequences of changes in plant mutualist and antagonist guilds in agriculture for selection on plant traits. I first explore how agriculture alters abundance and community structure of mutualist

pollinators and antagonist seed predators of wild *Helianthus annuus texanus*. Mutualists were more abundant near crops, whereas antagonists were more abundant far from crops near natural habitat. In addition, mutualist pollinator communities were more diverse near sunflower crops. Plant mutualists and antagonists respond differently to agriculture. Next, I explore how these changes in abundance and community structure of mutualists and antagonists influenced natural selection on *H. a. texanus* floral traits. Natural selection on heritable floral traits differed near versus far from crop sunflowers, and overall selection was more heterogeneous near crop sunflowers. Furthermore, mutualist pollinators and antagonist seed predators mediated these differences in selection. Finally, in Chapter 4, I ask if variation in interaction outcomes differs across types of species interactions. Furthermore, I examined the relative importance of factors that create context-dependency in species interactions. Using meta-analysis of 353 papers, we found that mutualisms were more likely to change sign of the interaction outcome when compared across contexts than competition, and predation was the least likely to change sign. Overall, species identity caused the greatest variation in interaction outcomes: *whom* you interact with is more important for context-dependency than *where* or *when* the interaction occurs. Additionally, the most important factors driving context-dependency differed significantly among species interaction types. Altogether, my work makes progress in understanding how species maintain interactions with multiple guilds of mutualists, how agriculture alters species interactions and subsequent natural selection, and the variation in species interaction outcomes and their causes.

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Chapter 1

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Chamberlain, S.A., & J.A. Rudgers. 2012. How do plants balance multiple mutualists? Correlations among traits for attracting protective bodyguards and pollinators in cotton(Gossypium). Evolutionary Ecology 26: 65-77

1.1. Abstract

Many species, both plants and animals, are simultaneously engaged in interactions with multiple mutualists. However, the extent to which separate traits that attract different mutualist guilds display negative or positive relationships remains largely unstudied. We asked whether correlations exist among extrafloral nectary traits to attract arthropod bodyguards and floral traits to attract pollinator mutualists. For 37 species in the cotton genus (*Gossypium*), we evaluated correlations among six extrafloral nectary traits and four floral traits in a common greenhouse environment, with and without correction for phylogenetic non-

independence. Across *Gossypium* species, greater investment in extrafloral nectary traits was positively correlated with greater investment in floral traits. Positive correlations remained after accounting for the evolutionary history of the clade. Our results demonstrate that traits to maintain multiple mutualist guilds can be positively correlated across related species and build a more general understanding of the constraints on trait evolution in plants.

1.2. Introduction

Mutualisms are ubiquitous in natural systems, and many species engage with more than one mutualist species simultaneously (Bronstein 1994; Mack and Rudgers 2008). Interactions involving a shared partner and more than one type of mutualist (i.e., interguild mutualisms) are common in nature, but have been little studied relative to pairwise and intraguild mutualisms (but see Stachowicz and Whitlatch 2005; Morris et al. 2007; Whitney and Rudgers 2009). For example, most angiosperms interact simultaneously with pollinators and mycorrhizal fungi, both of which acquire carbon from the plant (Gange and Smith 2005; Cahill et al. 2008).

Understanding how organisms allocate resources among guilds of mutualists is important for predicting constraints on the evolution of plant traits. Within species, the costs of rewarding mutualists could lead to trade-offs among investment in traits that affect different mutualist guilds. For example, in grasses that support both nutritional mutualists (mycorrhizal fungi) and protection mutualists (fungal endophytes), the presence of endophytes in leaves reduced the

rate of colonization by mycorrhizal fungi in roots (Omacini et al. 2006; Mack and Rudgers 2008). Through evolutionary time, such constraints could produce species that are specialized to reward a single mutualist guild. Alternatively, simultaneous interactions with two or more guilds of mutualists could lead to positive correlations among traits of the rewarding species, where investment in one mutualist guild is associated with increased investment in the other mutualist guild. Despite advances in our understanding of the correlated evolution of traits involved in antagonistic species interactions (Agrawal and Fishbein 2006; Agrawal 2011), we know little about the correlated evolution of traits that attract multiple mutualists. Whether traits are positively or negatively correlated has important implications for macroevolution (Agrawal 2011). For example, positively correlated evolution of plant defense traits is associated with adaptive radiation in *Asclepias* L. (Agrawal et al. 2009b). In addition, trait correlations can have ecological effects. For example, a trade-off between plant defense and plant growth among milkweed species (*Asclepias*) affected densities of insect herbivores (Mooney et al. 2010). As most species interact with multiple mutualists, correlations among traits to attract mutualists are likely to influence trait evolution.

Many plant species interact with both pollinating mutualists and protection mutualists. These plants invest in the production of nectar, pollen, and floral displays to attract insects and birds that move plant gametes (Klinkhamer et al. 1989; Waser et al. 1996; Brody and Mitchell 1997), while also producing nectar outside of the corolla (extrafloral nectar) to attract bodyguards, particularly ants, as a means of indirect defense against plant herbivores (Heil and McKey 2003). Plants

with extrafloral nectaries are not as common as those with floral nectaries, but worldwide, there are 113 angiosperm plant families with extrafloral nectaries, including approximately 4,000 plant species (K. Keeler, pers. comm.). There is much evidence that pollinators exert selection on floral traits (e.g., Benitez-Vieyra et al. 2006; Gómez et al. 2008; Young 2008), and while the body of evidence is smaller, ant bodyguards can also act as agents of selection on extrafloral nectar traits (Rudgers 2004; Heil et al. 2005; Rutter and Rausher 2007). Although pollinating and protection mutualists are generally associated with floral and extrafloral nectaries, respectively, this is not always true, potentially complicating correlations among these traits. For example, ant protective bodyguards that are most commonly associated with extrafloral nectaries can also feed on floral nectar or anthers. Prior work suggests that ants generally reduce plant fitness as a result of visiting flowers (Fritz and Morse 1981; Ness 2006; Galen and Geib 2007; Lach 2007; but see Schatz et al. 2006). This indirect cost of ants could result in reduced investment in floral traits for plants that are dependent on bodyguards, as a mechanism to limit ant visitation to flowers. What remains unclear is whether one set of traits could constrain plant investment in the alternate set.

Here, we took a comparative approach using 37 species of *Gossypium* L. (cotton; Malvaceae) grown in a greenhouse to ask whether there are trade-offs or positive correlations among plant traits used to attract bodyguard mutualists versus pollinating mutualists. Specifically, we addressed the following questions: (1) Across *Gossypium* species, do extrafloral nectary and floral traits display negative

correlations, suggesting trade-offs, or positive correlations? (2) Do *Gossypium* traits to reward mutualists vary among clades or geographic regions of origin?

1.3. Materials and Methods

1.3.1. Gossypium extrafloral and floral nectaries and their visitors

All *Gossypium* L. species except *G. tomentosum* Nuttall ex Seemann produce extrafloral nectaries on the veins of the abaxial leaf surface (foliar) as well as either subtending the bracts (calyx) or above the bracts (involucellar), and on the adaxial surface of sepals of buds, flowers, and fruits (Dejoode and Wendel 1992). Hereafter, we refer to calyx and involucellar nectaries collectively as bracteal nectaries. The number of foliar nectaries on a single leaf generally ranges from one to five. Both nectary types can vary in size, shape, and the volume of nectar produced. Visitors to extrafloral nectaries are largely ants, but may also include wasps, flies, lepidopterans, and other insects (see also Rudgers et al. 2010).

All *Gossypium* L. species produce floral nectar (Fryxell 1979). *Gossypium* L. flowers are either solitary or arranged in elongated sympodia, and each flower has five obovate, white to rose colored petals, often with a dark spot in the petal claw (Fryxell 1979). Flowers are hermaphroditic, and the androecium is composed of numerous stamens fused into a staminal column that surrounds the gynoecium (Fryxell 1979). The stigma is 3-5 lobed, and sometimes exceeds the anthers (Fryxell 1979; Kubitzki 2003); greater stigma exsertion is often correlated with greater outcrossing (Motten and Stone 2000). *Gossypium* L. flowers generally produce

nectar diurnally, except for two species: *G. longicalyx* J.B. Hutchinson & Lee and *G. tomentosum* Nuttall ex Seemann (Fryxell 1979). Nocturnal flowers of these species are likely visited by lepidopterans, rather than bees, which are considered the primary pollinators of *Gossypium* L. with diurnal flowers (Fryxell 1979).

The genus *Gossypium* L. includes eight monophyletic diploid genome groups (hereafter 'clades'; A, B, C, D, E, F, G and K) and one tetraploid genome group (AD, including all the cultivated species; Wendel and Cronn 2003; Wendel et al. 2010). Clades correspond roughly with geographic distribution: Africa (B, E, and F); the Americas (D and AD); Asia (A, E); and Australia (C, G, and K). *Gossypium* L. is thought to have undergone rapid, global radiation early on in the genus, with closely spaced divergence events in time facilitated by long-distance trans-oceanic dispersal (Wendel and Cronn 2003).

1.3.2. Study location and organisms

We conducted studies on 37 *Gossypium* L. species, with geographic distributions spanning five continents and all eight phylogenetic clades (Appendix Table A1). Traits were measured in greenhouses at the United States Department of Agriculture (USDA) Southern Plains Agricultural Research Center in College Station, Texas, USA (30°37'3" N, 96°21'38" W) between November 2008 and August 2009. The advantage of a common greenhouse setting was that differences in trait investment among species were not confounded with particular environmental conditions unique to each species' habitat (see also Cavender-Bares et al. 2004; Agrawal et al. 2009a). Plants ranged in age from 1-5 y ($\bar{X} \pm 1\ s.e.$ = 2.9 ± 0.2), had

produced significant woody biomass, and had reached reproductive maturity. Temperatures in the greenhouses ranged from 15.6-29.4°C ($\bar{X}$ = 21.1°C), humidity was ambient (range ~ 60-90%), and no supplemental lighting was used. Plants were grown from seed and represent a permanent germplasm collection maintained by the USDA.

1.3.3. Extrafloral and floral nectary traits

We obtained trait measurements on 1-3 individuals per plant species, effectively treating species as the unit of replication. This has been a common approach in studies across species (e.g., *n* = 5 in Agrawal and Fishbein 2006). Sample sizes per species were limited by the availability of plants in the permanent collection, and the large size at reproduction of some species required significant greenhouse space. The small number of individuals per species limits the precision of the estimate of the mean trait values for each species, but does not affect corrections for phylogenetic non-independence, because these methods analyze only the mean, not variance, within species. Methods are available for including variation among individuals within a species in independent contrasts (Ives et al. 2007; Felsenstein 2008), but we have only one individual for roughly half of our 37 species. More precise estimates of mean trait values would reduce variation in the data and increase the ability to detect pattern; thus, our methods make this study conservative if correlations among traits are detected, but cannot rule out the possibility that non-significant correlations could become significant with larger sample sizes.

We quantified six extrafloral nectary traits: the proportion of leaves with active extrafloral nectaries (number of leaves with active nectaries / total number of leaves), foliar extrafloral nectar volume, bracteal extrafloral nectar volume, foliar extrafloral nectary area, bracteal extrafloral nectary area, and foliar extrafloral nectar sugar concentration. All leaf traits were measured on randomly selected fully expanded leaves, to capture the range of variation present within a plant (Heil et al. 2000). Two traits, the proportion of leaves with extrafloral nectaries and extrafloral nectary area, have shown significant heritability and were under significant directional selection in one cotton species, *G. thurberi* Todaro (Rudgers 2004; Rudgers and Strauss 2004). We determined the proportion of leaves with extrafloral nectaries for 30 leaves per plant. We measured standing stocks of extrafloral nectar (all accumulated nectar produced on plants that were untouched) on three leaves per plant. Nectar was removed using glass microcapillary tubes (40 μL, 2 μL, or 1 μL) (Drummond Scientific Company, Broomall, PA, USA), and care was taken not to puncture plant tissue when removing nectar. We assessed nectar volume by measuring the length of fluid in the capillary to the nearest 0.01 mm using digital calipers. For a subset of 28 plant species, we also measured the rate of foliar nectar production 24 hr^{-1} on the same three leaves. Foliar extrafloral nectar production rate 24 hr^{-1} was positively correlated with standing stock (Pearson correlation, r = 0.67, P = 0.0001, n = 28 species; Appendix Fig. B1), suggesting that standing stock measurements provided a useful estimate of the species level trait of foliar extrafloral nectar production. After measurements were made for each plant, we removed leaves at the base of the petiole, transported them in a cooler, and stored

them at -20°C following methods in Rudgers et al. (2004). We defrosted leaves, scanned them using an HP Scanjet 5590 digital scanner, and converted to TIFF images, at either 600 or 2400 dpi, for measurements of leaf area and extrafloral nectary area, respectively. We determined the area of leaves and extrafloral nectaries to the nearest 0.001 mm with image analysis software (Scion Image, Scion, Fredrick, Maryland, USA). Standing stock nectar was positively correlated with extrafloral nectary size (r = 0.51, P = 0.001, n = 28) across species. We stored nectar in vials at -20°C until sugar concentration was quantified. Extrafloral and nectar samples were thawed and diluted with deionized water to measure sugar concentration (sucrose equivalents) using temperature-corrected hand-held refractometers (Eclipse Series, Regular and Low-volume 0-50° BRIX, Bellingham & Stanley Inc., Lawrenceville, GA, USA).

We measured four floral traits: floral nectar volume, flower size, stigma exertion, and floral sugar concentration. We measured standing stocks of floral nectar on up to two flowers per plant between 12:00 and 15:00. We assumed that standing stocks of floral nectar adequately reflected differences among species because flowers persist for only a few hours to one day. Maximum petal length, maximum petal width, and stigma exertion (distance from tip of stigma to uppermost anther) were measured to the nearest 0.001 mm using digital calipers without removing flowers from the plants. Finally, we also measured plant size as basal stem diameter (mm) to assess whether traits were size-dependent (see "Trait correlations" below; see also Rudgers 2004).

1.3.4. Trait correlations

We calculated Pearson correlation coefficients for all pairwise combinations of extrafloral nectary and floral traits using the cor.test function in R v.2.10.1 (R Development Core R Development Core Team 2008). For all analyses, we averaged trait values when more than one individual per species was sampled, such that species was the unit of replication. The proportion active foliar extrafloral nectaries was arcsine square-root transformed, and foliar extrafloral nectar volume, foliar extrafloral nectar sugar concentration, foliar extrafloral nectary area, bracteal extrafloral nectar volume, bracteal extrafloral nectary size, floral nectar volume, floral nectar sugar concentration, and flower size were log10 transformed prior to analysis to meet assumptions of normality. We performed corrections for multiple tests using sequential Bonferroni correction (Holm 1979) separately for each of four extrafloral nectary-floral trait combinations. Although species traits often scale with organism size, we found no significant correlations between plant size (basal stem diameter) and any of the 10 extrafloral nectar or floral traits (Pearson product-moment correlation, range of $r = -0.41$ to 0.34, all $P \geq 0.07$). Furthermore, using the residuals of each trait regressed on plant size did not result in qualitatively different results from traits not regressed on plant size. For simplicity, we present data that has not been corrected for plant size.

To incorporate phylogenetic relatedness, we calculated standardized phylogenetically independent contrasts (PICs) for all 10 traits using the *Gossypium* L. phylogeny (Fig. 1; see Phylogeny Reconstruction methods in Appendix C), then

analyzed the 24 pairwise extrafloral-floral trait correlations. We calculated Pearson product-moment correlation coefficients among trait contrasts, and we applied sequential Bonferroni corrections to reduce Type I error.

1.3.5. Ordination

To capture overall differences in trait investment among species, we conducted non-metric multidimensional scaling analysis in Primer v.6.1.10 (NMDS; Clark and Gorley 2007). We used five extrafloral nectary traits (proportion active foliar extrafloral nectaries, foliar extrafloral nectar volume [standing stock], foliar extrafloral nectary area, bracteal extrafloral nectar volume, and bracteal extrafloral nectary size) for the extrafloral nectary traits ordination, and three floral traits (floral nectar volume, flower size [maximum petal length X maximum petal width], and stigma exertion) for the floral traits ordination. Due to two negative values, we made stigma exertion values positive by adding the absolute value of the most negative stigma exertion value to all species. Foliar extrafloral nectar volume, bracteal extrafloral nectar volume, bracteal extrafloral nectary size, floral nectar volume, flower size, and stigma exertion were $\log_{10}+1$ transformed prior to analysis to improve ordination fit. Proportion active foliar extrafloral nectaries and foliar extrafloral nectary area were arcsine square-root transformed. We only used *Gossypium* L. species for which we had all available data for the eight traits. We excluded sugar concentration data due to low sample sizes of species with these traits. We also excluded three extreme outlier species that inflated the stress of the ordination and prevented a satisfactory NMDS solution: 1) *G. tomentosum* Nuttall ex

Seemann lacked extrafloral nectaries (i.e., zeros for all extrafloral nectary traits; and was also removed from the floral traits ordination for consistency), 2) *G. costulatum* Todaro had very large bracteal extrafloral nectary size (4,120% higher than the next largest species), and 3) *G. laxum* Phillips presented a low, outlying stigma exertion value. We standardized each trait to each trait's maximum. The final extrafloral nectary and floral traits ordinations were both run with 28 species (9,999 iterations, Bray-Curtis distance metric). We calculated Pearson's *r* for correlations between the traits and the NMDS axes to assess the influence of each trait on each axis.

1.3.6. Clustering traits within Gossypium clades and geographic regions of origin

We examined whether traits were clustered among the *Gossypium* L. clades in the NMDS using analysis of similarities (ANOSIM; Clark and Gorley 2007), which tested whether trait variation within each *Gossypium* L. clade designation was significantly smaller than variation between clades. We also tested for a geographic signal in extrafloral and floral traits (n = 3 groups: Asia, Australia, and the Americas). Statistical significance was assessed by permuting the grouping vector to obtain the distribution of the test statistic, R (a metric for the difference between the variation between groups and the variation within groups), under a null-model (Clark and Gorley 2007). We used a Bray-Curtis distance metric, with traits transformed as described in *Ordination* above. ANOSIM was run separately for extrafloral nectary and floral traits (9,999 permutations), with 28 species for each analysis. B and F clades were excluded from the test for clade effects, as each had

only one representative species. In the case of a significant *R*-value, we then examined pairwise tests of difference among individual clades or continents. If groups significantly differed, we identified the traits contributing most to differences among groups using SIMPER analysis, which ranks individual traits by their contribution to the partitioning of groups (Clark and Gorley 2007). Qualitatively similar results for these analyses were detected with PERMANOVA through the *adonis* procedure in R v.2.10.1 (R Development Core R Development Core Team 2008).

1.4. Results

1.4.1. Across Gossypium species, are there significant correlations between floral traits and extrafloral nectary traits?

There were no significantly ($P < 0.05$) negative correlations among extrafloral nectary and floral traits, rejecting the hypothesis of trade-offs among investments in pollinators versus bodyguards. Furthermore, four of the 24 pairwise correlations and seven of the phylogenetically independent contrast (PIC) correlations among traits were significantly positive (Table 1). After sequential Bonferroni correction, three and four correlation coefficients remained significantly positive in the raw data and in the PICs, respectively (Table 1). Although accounting for evolutionary history did not strongly alter insights into relationships among extrafloral nectary and floral traits, four floral-extrafloral nectar trait correlations did change. Specifically, four pairwise trait combinations, floral nectar volume and

proportion active foliar extrafloral nectaries, floral sugar concentration and proportion active foliar extrafloral nectaries, floral nectar volume and foliar extrafloral nectary area, and floral sugar concentration and bracteal extrafloral nectary area, changed from non-significant in the raw data analysis to significantly positive in the PICs analysis. In contrast, one pairwise trait combination, flower size and bracteal extrafloral nectary area, changed from significantly positive in the raw data analysis to non-significant in the PICs analysis.

Non-metric multidimensional scaling (NMDS) resulted in 2-dimensional solutions for both sets of mutualist-attracting traits (Fig. 2). In the extrafloral nectar trait NMDS, bracteal extrafloral nectary volume (r = 0.83) was most strongly positively correlated with Axis 1, while foliar extrafloral nectary volume was most strongly positively correlated with Axis 2 (r = 0.82; Appendix Table D1). There was no indication that species investing in foliar extrafloral nectar invested less in bracteal extrafloral nectar. In the floral trait NMDS, flower nectar volume was most strongly positively correlated with Axis 1 (r = 0.92), while stigma exertion (r = 0.85) and flower size (r = 0.64) were positively correlated with Axis 2 (Appendix Table D1). Thus, cotton species that produced more floral nectar also had larger flowers and greater stigma exertion (upper right quadrant Fig. 2b), possibly indicative of a suite of traits to enhance outcrossing.

Consistent with the positive correlations detected in the analysis of pairwise trait combinations, Axis 2 of the extrafloral nectary traits NMDS was significantly positively correlated with Axis 1 of the floral traits NMDS (Pearson's r = 0.60, P =

0.0009). The other NMDS axes were not significantly correlated (floral Axis 1-extrafloral Axis 1: r = -0.05, P = 0.798; floral Axis 2-extrafloral Axis 1: r = 0.06, P = 0.771; floral Axis 2-extrafloral Axis 2: r = 0.02, P = 0.920). These results reinforce the conclusion that foliar extrafloral nectary volume (as detected in pairwise analysis) was the key defensive mutualist trait that was positively associated with plants' investment in floral nectar.

1.4.2. Do Gossypium traits vary among clades or geographic regions?

In pairwise tests there was a difference between Australia and Asia (ANOSIM; R = 0.34, P = 0.039) in floral trait investment. A significant percentage of the difference between Australia and Asia was attributable to greater floral nectar volume (48%), larger flower size (27%), and greater stigma exertion (25%) for species from Australia, as detected by SIMPER analysis. There was no significant difference among *Gossypium* clades in the extrafloral nectary traits ordination (ANOSIM; R = -0.025, P = 0.570) or in the floral traits ordination when all clades were included in the analysis (ANOSIM; R = 0.09, P = 0.197). Additionally, there was no significant difference among geographic regions in the extrafloral nectary traits ordination (ANOSIM; R = 0.02, P = 0.373; Fig. 2a) or in the floral traits ordination (ANOSIM; R = 0.20, P = 0.014; Fig. 2b).

1.5. Discussion

Our work is one of a few studies to examine correlations among traits that attract multiple mutualist guilds for any taxon (see also Whitney and Rudgers

2009), and the only study, to our knowledge, to investigate relationships among traits mediating pollination and protection mutualisms. Specifically, our results showed positive interspecific correlations between traits to attract ant bodyguards and traits to attract pollinators in the genus *Gossypium*.

Positive correlations remained when we accounted for the phylogenetic relatedness of *Gossypium* species, demonstrating that the associations between plant rewards to pollinator and protection guilds are not driven by phylogenetic non-independence in the data. The positive correlations we observed for extrafloral nectary and floral traits are consistent with the results of Whitney and Rudgers (2009), who found positive interspecific correlations between plant investment in fruit traits (seed dispersal) versus floral traits (pollination). In a recent meta-analysis, Morris et al. (2007) showed that the interactive effects of multiple mutualists on plant performance were, on average, positive. Although Morris et al. (2007) did not include species traits in their analysis, plant performance is often correlated with trait investment (e.g., Poorter et al. 2008), supporting the results we found here. In a similar study, albeit on traits involved in antagonisms, Agrawal and Fishbein (2006) demonstrated positive correlations between physical trichome defenses and chemical latex defense traits across 24 species of milkweed (*Asclepias*). Altogether, these results suggest that plants may experience fewer investment trade-offs among different functional traits than previously assumed.

There are several potential mechanisms that may underlie the positive correlations in this study. Positive correlations among extrafloral nectary and floral

traits could be shaped by pleiotropy or tight linkage of genes underlying these traits. However, there is no evidence to date that genes for extrafloral nectary and floral traits are closely linked in any plant species (M. Heil, J. Wendel, pers. comm.), and further, we found little evidence for phylogenetic signal in either class of plant traits (see *Phylogenetic Signal* in Appendix C). Sequencing of a *Gossypium* genome is underway (Chen et al. 2007) and should help to inform whether genes for extrafloral nectary and floral traits are linked. Positive correlations among extrafloral nectary and floral traits suggest that the evolution of extrafloral nectaries and flowers may also be linked. For example, if selection were stronger on the traits of flowers than on extrafloral nectary traits, linked extrafloral nectary traits could be 'dragged along' with the evolution of flowers. Traits that are physiologically linked (e.g., extrafloral and floral nectar) may be more strongly correlated than traits that are not physiologically linked (e.g., extrafloral nectary size and flower size; Wright et al. 2007). Some evidence from our study supports this assertion. For example, of all the extrafloral nectary traits, foliar extrafloral nectar volume was most strongly correlated with floral nectar volume (Table 1). However, floral nectar volume was only weakly correlated with nectar volume in bracteal nectaries, which are located spatially closer to flowers than foliar extrafloral nectaries. Lastly, it may be that both sets of traits are evolving independently, through different selection pressures.

A few caveats deserve consideration. First, we measured extrafloral nectary and floral traits in a greenhouse. Although this method controlled for environmental variation, species were removed from their ecological contexts, where trait expression may be environmentally plastic. Second, despite correlations among

traits, we have no evidence that the floral or extrafloral traits have important ecological consequences for the majority of wild cotton species. However, the ecological context of extrafloral nectar traits has been well documented in *G. thurberi*: ant visits to plants were mediated by extrafloral nectar; plant benefits (reduced herbivory) increased with higher extrafloral nectar; plants with more extrafloral nectaries had higher fitness; and variation in plant fitness among populations corresponded to variation in ant abundance and community species composition (Rudgers and Gardener 2004; Rudgers and Strauss 2004). Third, although our statistical power is correctly based on species as replicates, we assumed that trait measurements on one to three individuals per species were adequate to represent each species. We argue that this effect makes any significant results conservative, provided that variation in species level traits is greater than variation within species, which appears to be the case for cotton (Eckstein et al. 1999; Rudgers et al. 2004; Hulshof and Swenson 2010). In addition, foliar extrafloral nectar volume was very similar between plants measured in the field (0.04 ± 0.01 s.e. μL, n = 9 plants) in Arizona (J.A.R., unpublished data) and in the greenhouse (0.05 μL, n = 1 plant). Last, if there is a trade-off between quantity and quality of extrafloral nectar, such that cotton species that produce greater quantity of extrafloral nectar have lesser quality extrafloral nectar, there may be a negative correlation between extrafloral nectar quality and floral traits. This possibility does not, however, negate the importance of the positive correlations between extrafloral nectar and floral nectar traits found here. The extent to which trait correlations

drive fitness differences among species depends on ant bodyguard and pollinator preference for and recruitment rates to extrafloral nectar and floral nectar traits.

1.6. Conclusion

We have documented positive correlations among plant traits for attracting multiple mutualist guilds (ant bodyguards and pollinators) in the cotton genus (*Gossypium*). Specifically, we have shown that extrafloral nectar traits to attract ant bodyguards and floral traits to attract pollinators are often significantly positively correlated, and never significantly negatively correlated. In addition, positive correlations between extrafloral nectar and floral nectar traits remained when accounting for phylogenetic non-independence among species. Our work builds a more general understanding of how multi-species mutualisms can shape the evolution of plant traits.

1.7. Acknowledgements

We thank J. Frelichowski and J. Love for facilitating research at the USDA Southern Plains Agricultural Research Center. J. Wendel advised on construction of the *Gossypium* phylogeny for use in phylogenetically independent contrasts. L. Seifert, L. Albert, and R. Ross collected data in greenhouses. R. Anwar and A. Gorischek provided laboratory assistance. J.A.R. was supported by the Godwin Assistant Professorship at Rice University.

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1.9. Tables

Table 1.1 Pairwise Pearson correlation coefficients for all extrafloral nectary (EF)-floral trait combinations.

Correlation coefficients (*r*) are given for raw data and for phylogenetically independent contrasts. Sample sizes (no. of species) are given parenthetically below correlation coefficients. Asterisks indicate *P*-values prior to correction for multiple tests. Those correlation coefficients remaining significant ($P < 0.05$) after sequential Bonferroni correction (Holm 1979) are shown in bold face type. *: P < 0.06; **: P < 0.05; ***: P < 0.01.

	Proportion active foliar EF		Foliar EF volume		Foliar EF sugar		Foliar EF area		Bracteal EF volume		Bracteal EF area	
	Raw	PICs	Raw	PICs	Raw	PICs	Raw	PICs	Raw	PICs	Raw	PICs
Floral nectar volume	0.34 * (31)	0.48** (29)	**0.58** *** (31)	**0.51** ** (29)	0.14 (16)	0.05 (14)	0.07 (30)	**0.59** *** (28)	0.05 (30)	-0.24 (28)	0.31 (28)	0.01 (27)
Flower sugar	0.06 (17)	0.73** (16)	0.28 (17)	0.19 (16)	0.30 (10)	.33 (8)	**0.63**** (17)	0.68 ** (16)	0.00 (17)	0.34 (15)	0.14 (17)	**0.80** *** (15)
Flower size	0.07 (30)	0.37* (29)	**0.48** *** (30)	**0.62** *** (29)	0.35 (15)	.02 (13)	0.35* (29)	-0.05 (28)	0.08 (29)	0.05 (28)	0.38 ** (28)	0.05 (27)
Stigma exertion	0.15 (30)	0.04 (28)	0.12 (30)	0.11 (29)	-0.12 (15)	0.26 (14)	0.14 (29)	-0.14 (28)	-0.22 (29)	-0.39* (28)	0.28 (28)	0.19 (27)

1.10. Figures

Figure 1.1 Bayesian phylogeny of the 37 Gossypium species (the outgroup *Ko* *drynarioides* is not shown to simplify the graphic).

Supports on branches are Bayesian posterior probabilities; all posteriors are show regardless of magnitude. Bar in bottom left represents the inferred number of substitutions per site. Clades and geographic regions are indicated at tips of the phylogeny prior to species names: Clades are A, B, C, D, E, F, G, K, and AD. Geograph regions are AF (Africa), AS (Asia), AU (Australia), NA (N. America), SA (S. America)

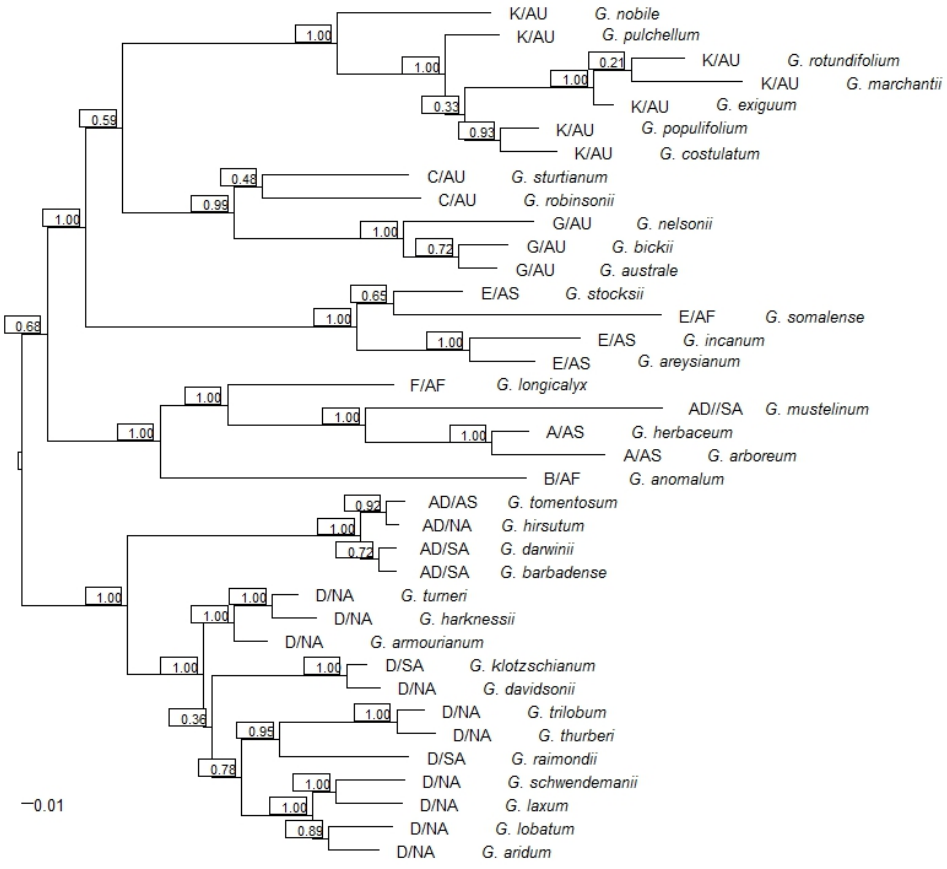
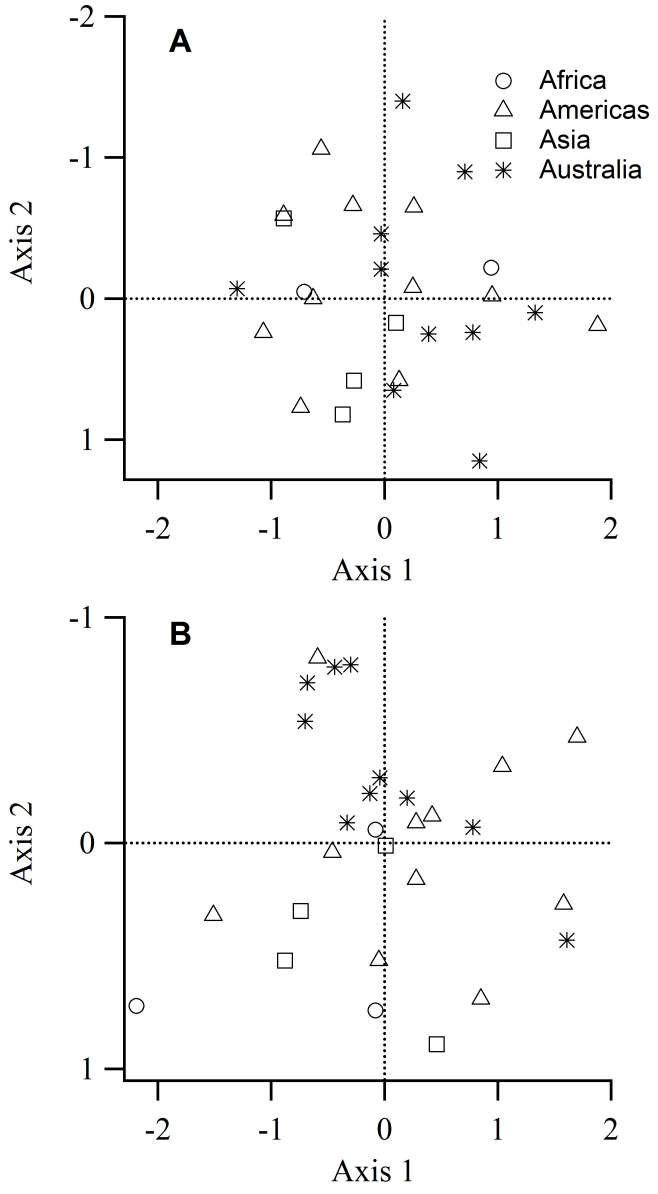


Figure 1.2 Two-dimensional NMDS ordination of Gossypium species defined by five extrafloral nectary traits (A) or three floral traits (B).

Clark recommends that stress values between 5 and 10 represent good ordinations with no real risk of drawing false inferences (Clarke 1993). Stress for the final NMDS solutions were 11 and 7 for extrafloral nectary and floral traits ordinations, respectively. See Appendix D for correlations of traits with NMDS axes.



1.11. Appendix A

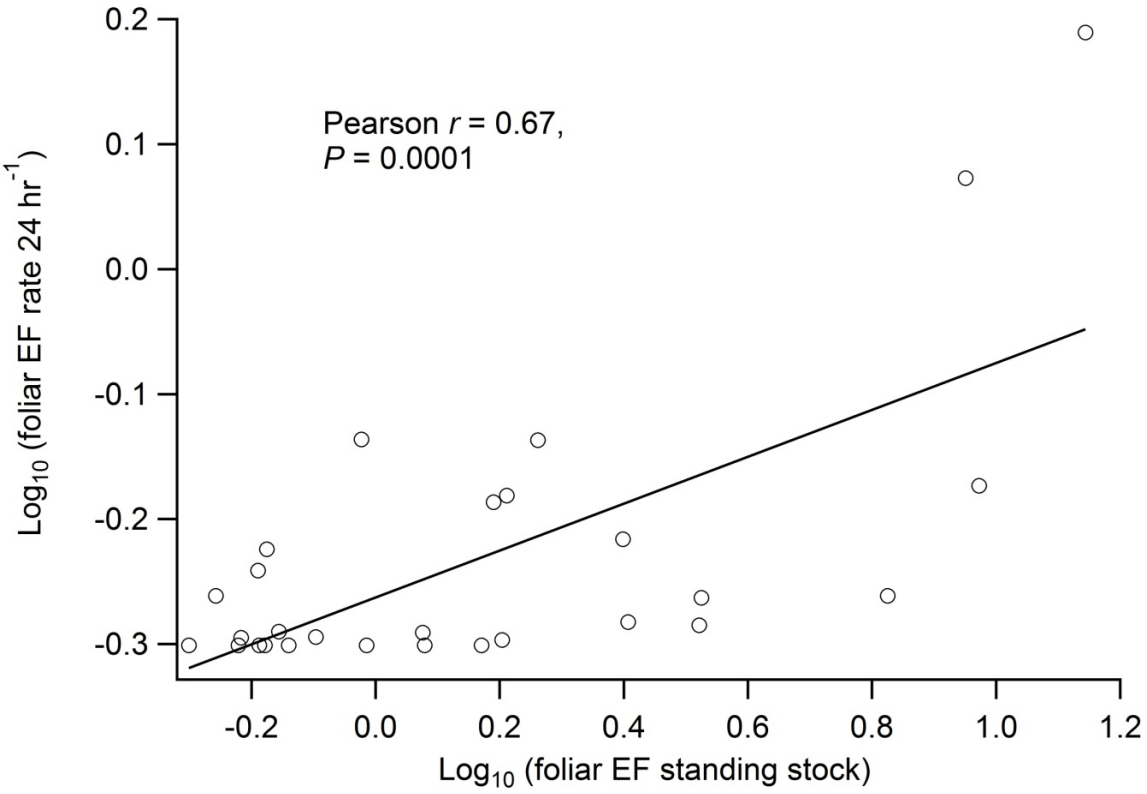
Table A1. Continental and regional distribution of the 37 *Gossypium* species and one outgroup species included in this study, and their accession numbers for ITS, AdhA, CesA1b, and ndhf genes. † indicates tetraploid species; the remainders are diploid. N=number of individuals used in analyses.

Species	N	Clade	Continent	Region	GenBank No.			
					ITS	*AdhA*	*CesA1b*	*Ndhf*
Kokia drynarioides	--	--	Asia	Hawaiian endemic	U56784	AF419965	AF419978	U55330
Gossypium anomalum	1	B	Africa	Africa	U56806	AF419961	AF419974	U55332
G. arboreum	2	A	Asia	Pakistan	U12712	--	--	U55331
G. areysianum	1	E	Asia	South Yemen	U56810	--	--	--
G. aridum	2	D	N. America	Colimia, Mexico	U12732	AF182135	AY699086	--
G. armourianum	1	D	N. America	Baja California, Mexico	U12725	AF182122	AY699090	--
G. australe	2	G	Australia	Western Australia	U56786	--	--	--
G. barbadense†	3	AD	S. America	Bolivia, Peru	U12715	AF085818	--	U55339
G. bickii	1	G	Australia	Northern Territory, Australia	AF057758	AF419964	AF419977	AF403555
G. costulatum	1	K	Australia	Northwestern Australia	U56790	--	--	--
G. darwinii†	1	AD	S. America	Galapagos Islands	U12716	--	--	--
G. davidsonii	2	D	N. America	Baja California, Mexico	U12729	AF182131	AY125071	AF520733
G. exiguum	1	K	Australia	Northwestern Australia	U56798	--	--	--
G. harknesii	1	D	N. America	Baja California, Mexico	U12727	AF182123	AY699091	--
G. herbaceum	2	A	Asia	India	U12713	AF136458	--	--
G. hirsutum†	2	AD	N. America	Mexico	U12719	AF090159	--	U55340
G. incanum	1	E	Asia	Yemen	U56811			

G. laxum	2	D	N. America	Guerrero, Mexico	U12730	AF182148	AY699097	--
G. lobatum	1	D	N. America	Michoacan, Mexico	U12731	AF182153	AY699099	--
G. longicalyx	1	F	Africa	Tanzania	U12722	AF419963	AF419976	U55338
G. marchantii	2	K	Australia	Western Australia	U56796	--	--	--
G. mustelinum†	2	AD	S. America	Brazil	U12714	--	--	--
G. nelsonii	1	G	Australia	Northern Territory, Australia	U56789	--	--	--
G. nobile	2	K	Australia	Northwestern Australia	U56797	--	--	--
G. populifolium	1	K	Australia	Western Australia	U56801	--	--	--
G. pulchellum	2	K	Australia	Northwestern Australia	U56802	--	--	--
G. raimondii	2	D	S. America	Peru	U12718	AF136459	--	U55335
G. robinsonii	1	C	Australia	Western Australia	U12710	AF136457	--	U55334
G. rotundifolium	1	K	Australia	Northwestern Australia	U56804	--	--	--
G. schwendimanii	1	D	N. America	Michoacan, Mexico	U12734	AF182141	AY125072	AF520734
G. somalense	2	E	Africa	North Africa	U56809	AF419962	AF419975	--
G. stocksii	2	E	Asia	Arabia	U56812	--	--	U55337
G. sturtianum	3	C	Australia	Australia	AF057753	--	--	--
G. thurberi	1	D	N. America	Arizona, USA	U12711	AF182126	AY699103	--
G. tomentosum†	2	AD	Asia	Hawaii	U12717	--	--	AF031577
G. trilobum	2	D	N. America	Western Mexico	U12723	AF182128	AY1235073	AF520735
G. turneri	1	D	N. America	Sonora, Mexico	U12726	AF182120	AY125074	U55336

1.12. Appendix B

Figure B1. Foliar extrafloral nectar (EF) production rate 24 $hr^{-1}$was positively correlated with foliar extrafloral nectar (EF) standing stock. Each data point is a *Gossypium* species (*n* = 28 species).



1.13. Appendix C

Supplementary methods and results on phylogeny reconstruction, trait evolution, and phylogenetic signal.

Phylogeny Reconstruction

We calculated phylogenetically independent contrasts (PICs; Felsenstein 1985) to examine correlations among traits while controlling for the evolutionary relatedness of *Gossypium* species using Mesquite v.2.6 software (Maddison and Maddison 2007). We reconstructed ancestral relationships of 37 *Gossypium* species using all currently available molecular data for *Gossypium* (as of May 2009), which included four genes: internal transcribed spacer regions of the nuclear ribosomal RNA genes (internal transcribed spacer; ITS1, ITS2, 5.8s), alcohol dehydrogenase A (AdhA), a cellulose synthase gene (CesA1b), and a plastid protein gene (NADH dehydrogenase subunit 5; ndhf). Gene sequences were downloaded from GenBank (Appendix S1). ITS1&2 were available for all species, while AdhA, CesA1b, and ndhf were available for 20, 15, and 13 species, respectively. *Kokia drynarioides* (Malvaceae) was used as the outgroup for all phylogenetic analyses. Gene sequences were aligned separately using BioEdit v.7 (Hall 1999), utilizing Clustal v.1.4 multiple sequence alignment with 10,000 iterations. After Clustal alignment, each gene alignment was manually scanned for errors. We concatenated all four genes using Mesquite's concatenate tool. We used alignments including all four genes together as well as each gene separately for all phylogenetic reconstructions. For each alignment, we estimated the most appropriate DNA substitution model using the

function *phymltest* within the ape package in R (R Development Core Team 2008); AIC values for each model were compared to pick the best model. For each of the five alignments, we used both Bayesian inference (MrBayes v.3.1.2; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) and maximum likelihood (ML) (PhyML v.3.0; Guindon and Gascuel 2003) for phylogenetic reconstruction. For Bayesian inference, we used default priors along with the DNA substitution model determined by *phymltest*. Posterior probabilities were determined with two runs, each using four MCMC chains and one million iterations. The first 25% of trees were omitted as burn-in before sampling the posterior distribution. For ML analyses, branch support was determined with 1000 nonparametric bootstrap replicates using identical parameters as the Bayesian analyses.

Comparison of the phylogeny used in analyses below (see Fig. 1 in the main text) with the phylogenies of each gene separately, both through Bayesian and maximum likelihood revealed that most nodes and clades are similar to the consensus tree presented in Fig. 1 (data not presented). Thus, we use the phylogeny with all four genes in our analyses. Furthermore, topological congruency, determined by the metric I_{cong} (de Vienne et al. 2007), was statistically significant between our phylogeny (see Fig. 1 in the main text) and that of other published phylogenies of *Gossypium* including Alvarez et al. (2005) (n = 13 *Gossypium* species; I_{cong} index = 1.53, P = 0.001), Small et al. (1998) (n = 8; I_{cong} = 1.47, P = 0.005), and Seelanan et al. (1997) (n = 24; I_{cong} = 2.1, P = <0.0001). Phylogenetically corrected trait correlations are more sensitive to tree topology than branch length estimations (see e.g., Diaz-Uriarte and Garland 1996).

Phylogenetic Signal

Methods

We calculated phylogenetic signal using Blomberg's *K* within the PICANTE package (Kembel et al. 2009) in R v.2.10.1 (R Development Core Team 2008). Larger values of *K* are equivalent to greater phylogenetic signal, whereas small *K* values represent little phylogenetic signal (Blomberg et al. 2003). We tested whether or not *K* values were significantly greater than *K* values generated from randomized trees. One-sided *P*-values were obtained by comparing observed *K* values to 9,999 randomized trees; $P < 0.05$ were considered significant. Due to missing data for some species, between 17 and 37 species were available for the analyses of phylogenetic signal. *K* should have relatively good statistical power (~0.8) for phylogenies with 20 or more species. However, two of our traits included only 17 and 20 species, suggesting that lack of statistical power may be responsible for lack of phylogenetic signal.

Results

Overall, EF and FL traits showed little phylogenetic signal (Blomberg's *K*). The only extrafloral nectar trait to show significant phylogenetic signal was bracteal extrafloral nectar volume ($K = 0.48$, $P = 0.023$, $n = 30$ species). Other extrafloral nectar traits showed no phylogenetic signal: proportion active extrafloral nectaries

(K = 0.24, P = 0.258, n = 37 species), foliar extrafloral nectary volume (K = 0.33, P = 0.239, n = 37 species), foliar extrafloral nectar sugar concentration (K = 0.33, P = 0.185, n = 20 species), foliar extrafloral nectary area (K = 0.19, P = 0.645, n = 36 species), and bracteal extrafloral nectary size (K = 0.17, P = 0.651, n = 30 species).

The only floral trait to show a marginally significant phylogenetic signal was stigma exertion (K = 0.41, P = 0.064, n = 30 species). Other floral traits showed no phylogenetic signal: floral nectar volume (K = 0.40, P = 0.190, n = 30 species), floral sugar concentration (K = 0.13, P = 0.948, n = 17 species), and floral size (K = 0.39, P = 0.210, n = 30 species).

References for Appendix C

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1.14. Appendix D

Table D1. Pearson correlation coefficients (*r*) for five extrafloral nectary and three floral *Gossypium* traits against the two axes of 2-dimensional NMDS ordinations (Fig. 2 in the text).

Trait	Axis 1	Axis 2
Extrafloral nectary traits ordination		
Proportion active extrafloral nectaries	-0.68	0.57
Foliar extrafloral nectary volume	-0.21	0.82
Foliar extrafloral nectary area	0.03	0.48
Bracteal extrafloral nectar volume	0.83	0.36
Bracteal extrafloral nectary size	0.25	0.61
Floral traits ordination		
Floral nectar volume	0.92	-0.29
Flower size	0.65	0.64
Stigma exertion	0.40	0.85

Chapter 2

2. Proximity to agriculture alters abundance and community structure of wild sunflower mutualists and antagonists

Scott A. Chamberlain, Kenneth D. Whitney, and Jennifer A. Rudgers

To be submitted to *Ecosphere* as an *Article*

2.1. Abstract

Anthropogenic modifications of the landscape, such as agriculture, are widespread globally and can reduce native biodiversity and homogenize communities by decreasing variation in species structure across sites. Partitioning anthropogenic impacts among species that have positive versus negative effects on plants may improve our ability to forecast the ecological and evolutionary consequences of these altered communities. Here, we manipulated the distance of populations of a wild sunflower species (*Helianthus annuus texanus*) to fields of its domesticated relative (crop sunflowers, *H. annuus*) and contrasted subsequent shifts in the abundance and community structure of

mutualists (pollinators) and antagonists (seed predators, folivores) of *H. a. texanus*. On average, populations of *H. a. texanus* near crop sunflowers supported significantly higher numbers of pollinators than those far from crop sunflowers, and pollinators were never greater far from crop sunflowers. In contrast, populations of *H. a. texanus* supported more seed predators when located far from crop sunflowers in the majority of cases. On average, folivore damage to plants was greater far from crop sunflowers, and was never greater near crop sunflowers. Contrary to the common idea that proximity to agriculture homogenizes community structure (β-diversity) we found that β-diversity was greater near crop sunflowers. Our results demonstrate that mutualists and antagonists of a wild plant respond differently to the proximity of a related crop species, indicating the potential for both altered population dynamics and complex selection pressures on wild species in agricultural landscapes.

2.2. Introduction

Human activities, such as urbanization, fragmentation, and the introduction of invasive species, can homogenize ecological communities by reducing variation in community structure across sites (McKinney 2006). Some evidence indicates that global agricultural intensification can also lead to biotic homogenization. For example, in Europe, increased pesticide use amplified similarities in both bee and hemipteran assemblages in agricultural relative to non-agricultural areas (Dormann et al. 2007). However, biotic homogenization is not the rule. Small mammal diversity did not vary across a gradient of arable land use intensity in conventional agricultural fields (Fischer et al. 2011).

Croplands, pastures, and rangelands constituted ~50% of the global vegetated land surface as of 2005 (Foley et al. 2005). Thus, increased proximity to agriculture has the potential to significantly impact the diversity of adjacent natural communities, and more specifically, may homogenize community structure relative to less modified habitats within in the landscape matrix.

Proximity to agriculture is likely to affect the abundance and community structure of functional groups in different ways. Plant mutualists and antagonists are two groups of organisms that are important for both crop productivity (as pollinators and pests) and the ecology and evolution of wild plants (Morris et al. 2007, Gómez et al. 2009). Reductions in plant antagonists on wild plants near crops could result from direct management of plant antagonists on crops (e.g., pesticides, tilling practices). Alternatively, reductions in plant antagonists could result from top-down effects from spillover of parasitoids. For example, greater parasitism of herbivores of wild mustard was correlated with increasing density of oilseed rape in the landscape (Gladbach et al. 2011). Alternatively, crop management practices could increase the abundance of plant antagonists on wild plants near crops if the antagonists respond to management by emigrating from crops to wild plants (Blitzer et al. 2012). In contrast, while farmers do not purposefully reduce plant mutualists, the management of antagonists could cause non-target declines, for example, in pollinators. For example, in a study of pollinators in Northeast Italy, pollinator abundance and diversity decreased at a number of spatial scales due to pesticides (Brittain et al. 2010). Alternatively, farmers may actively

supplement mutualist populations through activities such as importing bees or adding mycorrhizal fungi (Vanengelsdorp and Meixner 2010, Roy-Bolduc and Hijri 2011).

The abundances and community structures of mutualists and antagonists can have both ecological and evolutionary consequences for wild plants. Ecological effects will depend on the relative interaction strengths of mutualism versus antagonism. In a meta-analysis, Morris et al. (2007) showed that, on average, mutualists in isolation had smaller effects on plant performance than antagonists in isolation; however, their joint effects were positive for plant performance. Simultaneous selection on the same trait (ecological pleiotropy) should be common for traits that influence both plant mutualists and antagonists (Strauss and Irwin 2004). For example, patterns of selection on floral traits are likely to be more spatially variable if there are conflicting pressures from pollinators versus seed predators (e.g., Cariveau et al. 2004). In addition, conflicting selection pressures from mutualists versus antagonists can enhance phenotypic variation in natural populations relative to selection mediated by only one interaction type (Irwin et al. 2003, Siepielski and Benkman 2010). Because mutualists and antagonists both influence plant evolution, it is essential to document how agriculture not only alters biotic communities, but also specifically affects plant antagonists versus plant mutualists.

While the importance of changes in mutualist and antagonist communities is likely to vary among individual systems, the relatedness of the crop to the wild plant is likely a factor. When the crop and wild species are closely related, the ecological and evolutionary effects of community shifts on the wild plant are likely to be stronger than

for less closely related species. As many crop plants are cultivated in regions where their wild progenitors are abundant and diverse (as evidenced by crop-to-wild gene flow in many of the major crops; Ellstrand et al. 1999), this phenomenon is likely quite common. To our knowledge, no prior studies have examined how the proximity to agriculture affects both antagonist and mutualist communities on related wild plants.

Here, we investigated how proximity to agriculture affects the abundance and community structure of plant mutualists and antagonists using crop sunflowers (*Helianthus annuus*) and their wild relatives (*H. annuus texanus*). We asked the following three specific questions: 1) Do mutualists (pollinators) and antagonists (seed predators, folivores) differ in abundance near versus far from crop sunflowers? 2) Do mutualists and antagonists differ in community structure near versus far from crop sunflowers? 3) Does the beta diversity of mutualists and antagonists differ near versus far from crop sunflowers?

2.3. Materials and Methods

Study system

Cultivated *Helianthus annuus* and its wild congeners (sunflowers; Asteraceae) provide a tractable system for studying how agriculture alters mutualist and antagonist communities for wild plants. First, wild *Helianthus* commonly occur along the borders of crop sunflower fields (Burke et al. 2002). Second, in sunflower growing regions in the US, crop and wild sunflowers can overlap for several mo. across the season in flowering phenology (Chamberlain, pers. obs.), leading to high

potential for shared mutualists (pollinators) and antagonists (seed predators, folivores) between crop and wild sunflowers. Texas has 20 native *Helianthus* species, many of which produce viable, hybrid offspring with crop sunflowers (Whitton et al. 1997, Linder et al. 1998), an indication of shared insect pollinators. Third, a diverse biotic community interacts with wild and crop sunflowers. The pollinator communities of both crop and wild sunflowers include in sum several hundred species of bees (Hurd Jr. et al. 1980), with honeybees particularly dominant in crop sunflowers (Greenleaf and Kremen 2006). Furthermore, the wild species, *H. a. texanus*, is obligately outcrossing (Rieseberg et al. 1998), suggesting an important fitness effect of pollinators. Seed predators (mainly Diptera, Lepidoptera, and Coleoptera) attack both wild and crop sunflowers, and their species-specific damage to sunflower seeds is easily quantified (Whitney et al. 2006). Seed predators and herbivores can strongly reduce fitness for annual sunflowers (Cummings et al. 1999, Pilson 2000, Snow et al. 2003, Whitney et al. 2006).

Study sites and design

We used a factorial design in which we manipulated proximity of wild sunflowers to crop sunflowers and the wild sunflower seed source (2 proximity levels x 2 seed source levels). We collaborated with five Texas growers to locate planting sites adjacent to existing crop sunflowers. At all sites, we chose fields planted with Clearfield® sunflowers, which are not genetically modified, but have been artificially selected to be resistant to the imidazolinone herbicides (Sala et al.

2008). In 2010, we manipulated the proximity of *H. a. texanus* to crop sunflowers: Near (*H. a. texanus* population planted 10 m from the crop) or Far (population planted 2.5 km from any crop sunflower, bordering natural habitat and next to other crops [wheat, cotton, corn, sorghum]). Populations were replicated at each of five farms in TX (Fig. 1), and were planted with 80-100 greenhouse grown seedlings. Final plant abundance in each population differed from the starting number due to some plant mortality. The proximity treatment was crossed factorially with a seed origin treatment to enhance the generality of results. *H. a. texanus* seeds were collected from each of two sites in 2009 (Source 1: 30.3 N, 97.5 W ; Source 2: 30.2 N, 97.6 W). In 2011, we used the same design as 2010 (proximity treatment crossed with seed origin treatment), but replicated at two of the five farms (Sites 1 and 2; see Fig. 1).

H. a. texanus seedlings were obtained by nicking seeds with a razor blade and germinating them on damp filter paper in late February each year (2010 and 2011). Germinating seeds were kept in the absence of light at room temperature and were moved into the light after they produced fine root hairs. Approximately eight-day-old seedlings were transplanted into peat pellets (J30100 Super; Jiffy, Denmark) and grown in a greenhouse at Rice University for approximately four weeks before transplanting to the field (early- to mid-April). Plants were transplanted to the field early- to mid-April so that wild plants flowering overlapped that of crop sunflowers. Seedlings were watered in the field every three to five days by hand until they established (2-4 weeks).

Plant traits

To account for possible scaling of pollinator and seed predator abundance with plant size, we calculated plant volume at the end of the season by measuring height to the tallest inflorescence (to the nearest cm) and diameter of the stem at the base (to the nearest 0.1 mm) Plant volume was calculated as the volume of a cylinder ($\pi r^2 h$), where r is the radius of the stem at the base, and h is the height following (Whitney et al. 2006). On average, plant volume did not differ among near and far populations (ANOVA, $F_{1,434} = 0.08$, $P = 0.785$). We also recorded plant abundance by counting the number of plants per population that survived to reproduction. On average, plant abundance did not vary among near and far populations (Welch test, $t = -0.64$, $P = 0.530$).

Pollinators

We used two methods to quantify pollinator abundances in H. a. texanus populations. First, we sampled pollinators by direct observations on our study sunflowers, quantifying pollinator visitation rate. We randomly selected ca. 30 plants in each population to observe throughout the flowering season. We observed the 30 plants in each population for five minutes per plant, over four to six observation periods during the flowering period (May-September). A pollinator visit was recorded when we observed a visitor making contact with anthers, stigmas, or

both. Pollinators that could not be identified to species in the field were collected for identification in the lab. Pollinator abundance was standardized by observation effort (minutes), and totaled for each plant across observation dates; abundances did not vary with the slight variations in numbers of surviving wild sunflowers among plots (Pearson correlation of mean visitation per plant by number of plants per plot; r = 0.27, *P* = 0.181, n = 25 plots). Plants were used as the unit of observation. Pollinators were observed for a total of 5,140 and 4,250 minutes in 2010 and 2011, respectively. As a response variable, we used pollinator visits per inflorescence per minute (no. of visits to a plant / no. of inflorescences / minutes observed), which removes variation due to floral display and observation effort.

Second, we used the water bowl trap method, which is the most efficient method to capture maximum diversity of pollinators in general, and is the least prone to observer bias (Westphal et al. 2008). This method catches the subset of pollinators that visit H. a. texanus as well as pollinators that do not visit this plant species. We set out three to six bowls, each of which were one of three different colors (white, blue, yellow) that are known to attract different groups of pollinators (Wilson et al. 2008), at each of two dates throughout the flowering period each year. We filled bowls with water and few drops of soap to break surface tension. Bowls were collected after 48 hrs in 2010 and 24 hrs in 2011, samples were placed in 70% ethanol, and sorted to the lowest possible taxonomic level. Pollinator abundance was standardized by observation effort (hours and number of bowls), and data were pooled across dates by color to obtain one value per bowl color per population.

Morphospecies for observations and bowl traps were identified to the lowest taxonomic ranking following Michener et al. (1994) and Michener (Michener 2000).

Seed predators

We quantified the abundance of seed predators on all plants in each *H. a. texanus* population from seeds collected in mesh bags (8 cm x 8 cm, made from plastic mesh; DelStar Technologies, Delaware) on three to six inflorescences per plant. Bags were installed after pollination to allow enough time for seed predators to interact with the inflorescence, but before shattering (seed drop) occurred to prevent seeds from dropping to the ground (following methods of Whitney et al. 2006). We collected bagged inflorescences at the end of the season (September), after seeds had matured and plants had senesced. We pooled all inflorescences per plant, and then sub-sampled ~80 randomly selected seeds with x10 dissecting microscope to quantify species-specific damage for *Neolasioptera helianthi* (Diptera: Cecidomyiidae), *Isophrictis* sp. (Lepidoptera: Gelechiidae), and *Smicronyx sordidus* (Coleoptera: Curculionidae). A total of 255,593 and 68,370 seeds were scored in 2010 and 2011, respectively. Total numbers of seeds damaged per plant were extrapolated using the number of inflorescences per plant. Number of seed predators was calculated assuming that each damaged seed was caused by a unique predator individual. This is clearly the case for *Neolasioptera* and *Smicronyx*, but this method may have overestimated abundance of *Isophrictis*, as individual *Isophrictis* larvae can damage multiple seeds. As a response variable for each seed predator

species for all analyses, we used abundance scaled to available resources, or proportion of attacked seeds per plant (seeds attacked / total seeds produced), which removes variation due to the size of the resource.

Folivores

We quantified folivory abundance for both chewing herbivore and herbivores that cause leaf vascular tissue damage assuming leaf damage was predictive of folivore abundance. Insect damage to leaves was recorded once for each plant in late May 2010. Folivory at this early stage in plant phenology (mean leaves per plant ± 1 SEM: 8.1 ± 0.1, range: 1-17) should influence plant fitness more so than folivory later in the season after inflorescences and seeds have been produced. We scored damage on the three oldest non-senescent leaves per plant. Damage scored was chewing damage (due to Orthoptera, Lepidoptera, and Diptera) and leaf vascular tissue damage (due to Hemiptera). Damage was scored visually for each leaf in the field by assigning a score from 0 to 4: 0 = no damage; 1 = low (ca. 1-5%) damage; 2 = medium (ca. 6-20%) damage; 3 = medium-high (ca. 21-50%) damage; and 4 = high (ca. 51-100%) damage. These scores were used to calculate a damage metric for each plant following Whitney et al. (2006):

$$D = \sum_{i=1}^{4} \frac{n_i(C_i)}{N},$$

where i is the damage category, n_i is the number of leaves in the ith category, C_i is the midpoint of each damage category (e.g., C_4 = 75.5%), and N is the total number of leaves surveyed per plant (range 1-3). Units for D are percent leaf area damaged.

Data analysis

Abundance

We analyzed abundance data for 2010 for Sites 1 through 5 separately for pollinator abundance (observational data), each seed predator species, and chewing and leaf vascular damage folivores. For all models, we used a mixed model, with effects of site, proximity to crop sunflowers (near vs. far), and their interaction as fixed effects, plant volume as a covariate, and population nested within site and proximity as a random effect. As we had some missing site X proximity X seed origin populations due to accidental destruction and wild pig damage, we did not include seed origin in these models. For pollinators, all three seed predator species, and folivores, we also modeled their abundance across years (2010 and 2011) for the two sites for which data was collected in both years (Site 1 and Site 2; see Fig. 1). We used the same models as above, but with year, and interactions, as additional factors. In all models, and models below for pollinator abundance in water bowl traps, we performed planned contrasts to compare the response between near and far from crop sunflowers within each site. Given many zeros in pollinator and seed predator abundance data, and folivore abundance data, model residuals never met

assumptions of parametric models. Therefore, we used randomization test equivalents of ANOVA to analyze abundance data for pollinators, seed predators, and folivores. Distribution-free randomization tests create an expected distribution of the p-value under the null hypothesis by randomizing the response variable on the independent variables, and calculating the test statistic and associated p-value (9999 runs). Then, the observed value is compared to the expected distribution generated by the randomization procedure. We used PROC MIXED within the SAS randomization-test macro program (SAS v.9.3, SAS Institute Inc., Cary, NC, USA; Cassell 2002).

We assessed whether pollinator abundance (as measured by bowl trapping) differed by proximity to crop sunflowers, site, and year. We ran separate models for 2010 using Sites 1 through 5, including the factors of site, proximity, the interaction between site and proximity, bowl color, and plot (nested within site and proximity) as a random effect. Replicates were bowl colors within each population, as bowl trap data were pooled across dates for each of three bowl colors (white, yellow, blue). We used planned contrasts to test differences in pollinator abundance Near vs. Far from crop sunflowers with the expectation that pollinator abundance should be greater near crop sunflowers. We also analyzed the response of abundance across years (2010 and 2011) for the two sites for which data was collected in both years (Site 1 and Site 2; see Fig. 1). We used the same models as above, but with year, and interactions between site and proximity, and the 3-way interaction (year X site X proximity) as additional factors. For water bowl trap pollinator data, we used mixed effects ANOVA.

Community structure

We conducted nonmetric multidimensional scaling analyses (NMS) to assess differences among sites and treatments in mutualist (pollinators) and antagonist (seed predators) community structure. In the NMS, we used the Bray-Curtis distance measure, and 9,999 iterations using the vegan package (R Development Core Team 2011, Oksanen et al. 2012). For pollinators, we used only the pollinator visitation data (there were insufficient data to run NMS for the bowl traps). For both pollinators and seed predators, we ran models with 2010 and 2011 data combined. We removed all morphospecies that were not represented in at least 5% of samples (McCune and Grace 2002). We used permutational multivariate analysis of variance (PERMANOVA; function adonis in the vegan package in R; McArdle and Anderson 2001, Oksanen et al. 2012) to test for differences in species assemblages due to year, site, proximity to crop sunflowers, and their interactions. Seed source was not included as a factor in the model because we were not specifically interested in its effects, and sample sizes were not large enough to include it. We used SIMPER (similarity percentages) analysis to identify the morphospecies that contributed most to differences between treatments and sites (Oksanen et al. 2012).

We expected that β-diversity of mutualists and antagonists would be greater far from crop sunflowers due to a greater diversity of crop types and being adjacent to natural habitat. To test whether β-diversity for mutualist and antagonist communities was greater among sites far from versus near crops we used

permutational analysis of homogeneity of group dispersions (also known as PERMDISP; function betadisper in the vegan package in R; Anderson 2006, Oksanen et al. 2012) to test for heterogeneity in community structure. Whereas PERMANOVA tests for differences in means of treatment levels in a distance matrix, PERMDISP tests for differences in dispersion from the centroid of treatment levels. Analyses were done using R v.2.14.1 (R Development Core Team 2011).

2.4. Results

1) Do mutualists and antagonists differ in abundance near vs. far from crop sunflowers?

Mutualists

On average, pollinator visitation to wild sunflowers tended to be greater near crop sunflowers than far from the crop. However, the effect of proximity to crop varied among sites and years. For pollinator visitation data in 2010, floral visitation rate was, on average, 137% greater near crop sunflowers (Fig. 2a) relative to far from crop sunflowers ($P = 0.027$; Table 1), but did not differ among sites ($P = 0.571$). The effect of proximity to crop sunflowers on floral visitation did not depend on the site (site X proximity: $P = 0.696$). However, we lost entire plots at some sites, suggesting that effects could be different if test were done within each site. Thus, we ran models for each site individually asking if floral visitation rate differed by proximity. Floral visitation rate was greater near crop sunflowers at Site 2 (111% greater Near), and at Site 3 (146% greater Near), but did not significantly differ with

proximity at Sites 1 or 5 (Site 4 was not tested due to low sample size; Fig. 2a); abundance tended to be greater near to relative to far from crop sunflowers at Sites 1 and 5.

For Sites 1 and 2, at which experiments were replicated in two years (2010-2011), floral visitation rate was on average 101% greater near crop sunflowers relative to far from crop sunflowers ($P < 0.003$; Table 1). Whether floral visitation rate differed by proximity varied by site ($P = 0.009$). In 2011, floral visitation rate was significantly greater near crop sunflowers at Site 1 (363% greater Near), but not at Site 2 (11% less Near). The lack of a difference in abundance at Site 2 in 2011 may relate to climate; 2010 was a relatively wet year across Texas, but 2011 was an extreme drought year; the drought was more severe near Site 2 relative to Site 1 (SAC, personal observation). Floral visitation rate did not differ among years or sites, and floral visitation rate at each site did not differ among years (Table 1). The effect of proximity to crop sunflowers on floral visitation did not depend on year, or site and year (Table 1).

In 2010, pollinator abundance in bowl traps did not vary with proximity from crop sunflowers ($F_{1,9} = 0.2$, $P = 0.675$), but abundance differed among sites ($F_{4,9} = 5.0$, $P = 0.021$) with the highest abundance at Site 1 which was ca. 1400% greater than abundance at the site with the lowest abundance (Site 4; Fig. 2c). There was no interaction between site and proximity ($F_{4,9} = 1.0$, $P = 0.454$). For Sites 1 and 2, at which experiments were replicated in two years (2010-2011), pollinator abundance did not differ by proximity to sunflower crops ($F_{1,10} = 0.09$, $P = 0.766$), but was on

average 420% greater in 2010 than 2011 ($F_{1,10}$ = 14.0, P = 0.004), and was 344% greater at Site 1 than Site 2 ($F_{1,10}$ = 10.9, P = 0.008). No interaction terms were significant. Floral visitation data and bowl trap data were positively correlated (Pearson correlation coefficient; r = 0.43, P = 0.033, df = 23).

Antagonists

Seed predators. Among the three seed predator species, the most abundant were Neolasioptera helianthi midges (mean % seeds attacked across individual plants in 2010 = 3.6%), followed by Isophrictis sp. moths (1.03%), then Smicronyx sordidus weevils (0.07%). On average, seed predators were more abundant far from crop sunflowers relative to near them, and more abundant in 2011 than 2010.

In 2010, *N. helianthi* abundance was, on average, 524% greater far from crop sunflowers (P = 0.007; Table 1, Fig. 3a). However, whether *N. helianthi* abundance differed by proximity to crop sunflowers depended on the site (site X proximity; P = 0.038); abundance was greater far from crop sunflowers at Sites 1 (418% greater Far) and 3 (1892% greater Far), but was 69% greater Near at Site 5. *N. helianthi* abundance did not significantly differ by proximity at sites 2 or 4 (Fig. 3a). In the analysis of sites 1 and 2 across years (2010 and 2011), *N. helianthi* abundance differed by proximity to crop sunflowers (P < 0.001), but in models for individual sites, there were no differences between Near and Far within each site (Fig 3d). *N. helianthi* abundance was 402% greater in 2010 than 2011 (P = 0.006), but did not differ among sites (P = 0.092). *N. helianthi* abundance depended on proximity to

crop sunflowers and year (proximity X year; $P = 0.010$); abundance at Site 1 was 418% higher far from crop sunflowers in 2010, but did not differ by proximity to crop sunflowers in 2011 (Fig. 3 a,d). Abundance at Site 2 did not differ by proximity in 2010 or 2011.

In 2010, *Isophrictis* sp. abundance was, on average, 940% greater far from crop sunflowers relative to near them ($P = 0.016$; Table 1, Fig. 3b). Whether *Isophrictis* sp. abundance differed by proximity to crop sunflowers did not depend on the site ($P = 0.109$). In comparison across years, Isophrictis sp. abundance but did not differ by proximity to crop sunflowers ($P = 0.089$), but in models for individual sites, abundance was 60% greater far from crop sunflowers relative to near at Site 2 (Fig. 3e); there was no difference between near and far at Site 1.

In 2010, *S. sordidus* sp. abundance, on average, was 3% greater near crop sunflowers relative to far from them ($P = 0.046$; Table 1, Fig. 3c), and there was no proximity X site interaction ($P = 0.538$). In comparison across years for Sites 1 and 2, whether *S. sordidus* abundance differed by proximity to crop sunflowers depended on the site (site X proximity; $P = 0.042$). *S. sordidus* abundance did not differ by proximity to crops at Site 1 in 2010, but was greater Far from crop sunflowers in 2011 (Fig. 3d). In addition, abundance was greater near crop sunflowers in 2011, but likely did not differ by proximity in 2010 (could not be statistically tested due to low sample size; see Fig. 3d). *S. sordidus* abundance differed by sites on average; abundance was 229% greater at Site 1 relative to Site 2 ($P = 0.034$).

Folivores. Similar to the pattern for seed predation, both chewing and vascular leaf tissue damage tended to be greater (by 65% and 124%, respectively, both $P < 0.001$) far from crop sunflowers than near them (Fig. 4). Chewing and vascular damage differed among sites, with chewing damage highest at Sites 4 and 5, and vascular damage highest at Sites 3 and 5 ($P < 0.001$). Whether damage differed by proximity to crop sunflowers varied with site (site X proximity: chewing and vascular damage, $P < 0.001$). Chewing damage was greater far from crop sunflowers at Sites 1 (252% greater Far) and 3 (165% greater Far) and 5 (23% greater Far), but did not significantly differ by proximity at sites 2 or 4 (Fig. 4a). Vascular damage was greater far from crop sunflowers at Sites 3 (74% greater Far) and 5 (246% greater Far), but did not differ by proximity at sites 1, 2, or 4 (Fig. 4b).

2) Does mutualist and antagonist community structure differ near vs. far from crop sunflowers?

Mutualists

Pollinator community structure varied with proximity to crop sunflowers (permutational MANOVA; $F = 3.02$, $P = 0.004$; Fig. 5a), among years ($F = 8.47$, $P = 0.0001$), and among sites ($F = 2.02$, $P = 0.004$). No interaction terms were significant (i.e., year X proximity, year X site, proximity X site, year X proximity X site). *Diadasia enavata, Halictus ligatus*, Apidae sp. 1, and *Megachile* sp. 1 all contributed at least 10% to dissimilarity between *H. a. texanus* populations near and far from crop

sunflowers, contributing 21%, 13%, 11%, and 10% to dissimilarity, respectively (Table A1).

Antagonists

Seed predator community structure differed by proximity to crop sunflowers (permutational MANOVA; $F = 4.55$, $P = 0.008$; Fig. 5b), among years ($F = 13.83$, $P < 0.001$), and among sites ($F = 2.40$, $P = 0.017$). Whether community structure differed by proximity to crop sunflowers depended on the site ($F = 2.25$, $P = 0.024$); no other interactions terms were significant (i.e., year X proximity, year X site, year X proximity X site). Isophrictis sp. contributed the most (50%) to dissimilarity between near and far from crop sunflowers, while Neolasioptera helianthi midges contributed slightly less to dissimilarity (46%) (SIMPER analysis; Table A2). Smicronyx sordidus weevils were a distant third in contribution to dissimilarity between populations near and far from crop sunflowers, contributing only 5% to dissimilarity.

3) Does beta diversity differ near vs. far from crop sunflowers?

Dispersion of communities from the centroid in an ordination measures the extent to which communities differ among sites; this can be thought of as β-diversity. We asked if β-diversity differed near versus far from crop sunflowers, with the prediction that β-diversity should be greater far from crop sunflowers

because the communities associated with the same crop should be homogenous compared to communities adjacent to diverse crops near natural habitat. Mutualist pollinator community dispersion was significantly greater near crop sunflowers relative to far from them (PERMDISP; mean distance to centroid [Far: 0.52; Near: 0.58]; $F = 6.80$, $P = 0.017$). Antagonist seed predator community dispersion was not different near versus far from crop sunflowers in ($F = 0.10$, $P = 0.745$).

2.5. Discussion

Agriculture covers nearly 50% the global vegetated land surface (Foley et al. 2005). Because of this, there is increasing interest in the extent to which agricultural landscapes alter biodiversity (Vellend et al. 2007, Ekroos et al. 2010). This study adds a new perspective to the study of biotic communities in agricultural landscapes through the lens of plant mutualists and antagonists. Mutualists and antagonists have opposing effects on crop and wild plants, and are managed differently in agricultural landscapes. Partitioning their responses to agriculture has potential to inform our understanding of the ecology and evolution of agro-ecosystems. We showed that abundance of mutualists and antagonists responded in opposite directions to proximity to crop sunflowers: Overall, mutualist pollinators increased in abundance near crop sunflowers, while antagonist seed predators and folivores decreased in abundance near crop sunflowers. In addition, β-diversity of mutualist pollinators was greater among populations planted near relative to far from crops in one of two years.

Abundance

We showed that abundance of mutualist pollinators increased near crop sunflowers relative to far from crop sunflowers. This result is consistent with at least two studies. First, Hanley et al. (2011), found that bumble bee (*Bombus* spp.) pollinators in England increased in abundance on hedgerows near flowering crops (e.g., beans), but did not increase in abundance on hedgerows along non-flowering crops (e.g., wheat). Second, Westphal et al. (2003) showed that the abundances of species of *Bombus* at many sites in Germany were positively related to the density of flowering crops in the landscape, but were not related to the presence of natural habitat. Our result is consistent with a potential mechanism: crop sunflowers provide a large pulse of resources (pollen and nectar), attracting many pollinators, with subsequent spillover onto flowering plants adjacent to crop sunflowers. A consequences of this result for wild plants in agricultural landscapes are reduced pollen limitation near flowering crops, which can alter natural selection on floral traits of wild plants (Sandring and Agren 2009; Chamberlain et al., unpubl. data), and increase population growth (Law et al. 2010).

Contrary to mutualists, antagonist seed predators and folivores were on average more abundant far from crop sunflowers. These results are contrary to findings of McKone et al. (2001), who reported that corn-rootworms were more abundant on wild sunflowers in prairie remnants near corn fields relative to far from corn fields. However, McKone et al. was an observational study, and examined

a crop not related to the wild species, which likely associates with a different biotic community. Our finding is consistent with two possible mechanisms. First, suppression of crop pests via pesticides (killing those on plants) and tilling (killing those in the soil) may decrease seed predator populations, thereby decreasing them on nearby wild sunflowers (Gladbach et al. 2011). Second, crop sunflowers could be a more attractive resource than wild sunflowers for seed predators, thereby reducing their populations on nearby wild sunflowers. Consistent with this idea, Blitzer et al. (2012) reviewed published studies and found that the flow of herbivores from wild to crop plants is much more common than the other direction.

Community structure

Despite evidence that biotic communities in agricultural landscapes are decreasing in both α- and β-diversity (Dormann et al. 2007, Ekroos et al. 2010), a framework for linking the consequences of this pattern to effects on wild plants in agricultural landscapes has been largely absent from the literature. We found that mutualist pollinators and antagonist seed predators differed in community structure near relative to far from crop sunflowers. β-diversity among pollinator communities was greater near relative to far from crop sunflowers, whereas β-diversity of antagonist seed predators and folivores did not differ by proximity to crop sunflowers. This finding is opposite of the common expectation that biotic communities should be homogenized when farther away from natural habitat, and especially near the same crop. This contradiction may arise from two factors. First,

the focal crop in our study was conspecific with the wild plant in which we examined subsequent changes in biotic communities. This means that they likely share many biotic interactions (see *Wild-crop relatedness* below). Second, crops provide an enormous pulse of resources, which often increases mutualist pollinators in the landscape (Westphal et al. 2003). Although a single crop is a homogenous environment, the resource pulse may lead to a more diverse community relative to nearby natural habitats since more resources support more diverse communities (Gillman and Wright 2010). The difference in β-diversity associated with wild plants near crops versus far from them in natural habitat likely changes with land use intensity. In intense agricultural landscapes, massive resource pulses have a greater effect on biotic communities relative to when they occur in less intensive agricultural landscapes (Westphal et al. 2003).

Differences in community structure near versus far from crop sunflowers may have important consequences for wild plants in agricultural landscapes. For example, less β-diversity among communities of plant mutualists could alter evolutionary trajectories of the plants with which they interact and affect the geographic mosaic of coevolution on the landscape scale (Siepielski and Benkman 2010). In another example, differences in community structure of ant bodyguard mutualists was at least partly responsible for among site variation in selection on extrafloral nectaries, a trait that attracts ant bodyguards (Rudgers and Strauss 2004).

Wild-crop relatedness

Our results may be the most general in contexts in which crops and their wild relatives coexist, as in *Helianthus annuus* in North America. Many crops are grown where related native species occur, including wheat in the Middle East, corn, squash, and peppers in Mexico, and potatoes from the southwestern USA (AZ and NM) to Uruguay (Jarvis et al. 2008). When the focal species or community of interest is unrelated to the crop we may expect different results. Unrelated native plant species are not subject to gene flow from crops, so native plants will be largely affected by the biotic community associated with different crops (assuming agricultural management equal across crop types). Since species interactions are evolutionarily conserved (Gómez et al. 2010), affects on native plants are likely to be greatest when growing adjacent to their closest crop relatives. That is, very distantly related native and crop plants will not on average interact with many of the same mutualists and antagonists

The mutualist-antagonist framework

Plant mutualists and antagonists are managed quite differently in agriculture. Whereas plant mutualists are not managed (mycorrhizae) or are supplemented (honeybees), plant antagonists are aggressively suppressed. We have shown that two groups of organisms, plant mutualists and antagonists, respond differently to agriculture. Because mutualists and antagonists have different ecological and evolutionary effects on wild plants, our results suggest that

populations of wild plants near their crop relatives versus far from them in natural habitat will lead to diverging populations. In addition to wild plants, a mutualist-antagonist framework could inform farm management. That is, mutualist pollinators of wild sunflowers, which are also weeds in crop fields (Kane and Rieseberg 2008), in this study were more abundant near crop sunflowers. Thus, suppression of weeds that are most likely to benefit from proximity to a crop relative be prioritized near their crop relatives versus farther from them. Despite these insights, more work needs to be done to make the mutualist-antagonist framework more general. Specifically, further work should consider other mutualists and antagonists that interact with plants besides those examined here. For example, soil communities are negatively affected by agriculture (Postma-Blaauw et al. 2010), which in turn could affect ecology and evolution in wild plants.

2.6. Conclusion

Our results suggest that a mutualist-antagonist framework can lead to useful insights into how plant-associated insect communities are altered in agricultural landscapes. Mutualist pollinators were more abundant on wild sunflower plants near crop sunflowers, while antagonists were more abundant far from crop sunflowers. Community structure of both mutualists and antagonists differed near versus far from crop sunflowers, although the proximity to crop sunflowers changed β-diversity of mutualists but not antagonists. Not only do agricultural landscapes alter biotic communities relative to those in pristine landscapes (Dormann et al. 2007), but our

results show that plant mutualists and antagonists can respond differently to the proximity to crops. Alteration of plant mutualists and antagonists in agricultural landscapes may have important consequences for ecology and evolution of wild plants.

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2.9. Tables

Table 2.1 Results of abundance analyses for pollinator mutualists and antagonists (seed predators and folivores).

Only *P*-values are presented because all analyses were randomization equivalents of ANOVAs (9999 iterations). Significant results ($P < 0.05$) are shown in bold; marginally significant results ($P < 0.06$) are italicized.

Variable	Pollinators	*N. helianthi*	*Isophrictis* sp.	*S. smicronyx*	Chewing folivores	Leaf vascular folivores
2010						
Site (S)	0.571	0.218	0.136	0.165	0.096	**0.009**
Proximity (P)	**0.027**	**0.007**	**0.016**	**0.046**	0.208	*0.053*
S x P	0.696	**0.038**	0.109	0.538	0.374	0.154
Plant volume	0.878	0.832	0.195	**0.010**	0.223	0.936
2010/2011						
Year (Y)	0.452	**0.006**	0.157	0.097	--	--
Site (S)	0.073	0.092	0.878	**0.034**	--	--
Proximity (P)	**0.003**	**0.035**	0.089	0.171	--	--
Y x S	0.143	0.449	0.688	0.256	--	--
S x P	**0.009**	0.386	0.640	**0.042**	--	--
Y x P	0.782	**0.010**	0.183	0.241	--	--
Y x S x P	0.147	0.239	0.218	0.080	--	--
Plant volume	**0.025**	**0.044**	**0.027**	0.947	--	--

2.10. Figures

Figure 2.1 Map of study sites in 2010 and 2011. Note that five sites were used in 2010, and only two of the sites used in 2010 were also used in 2011.

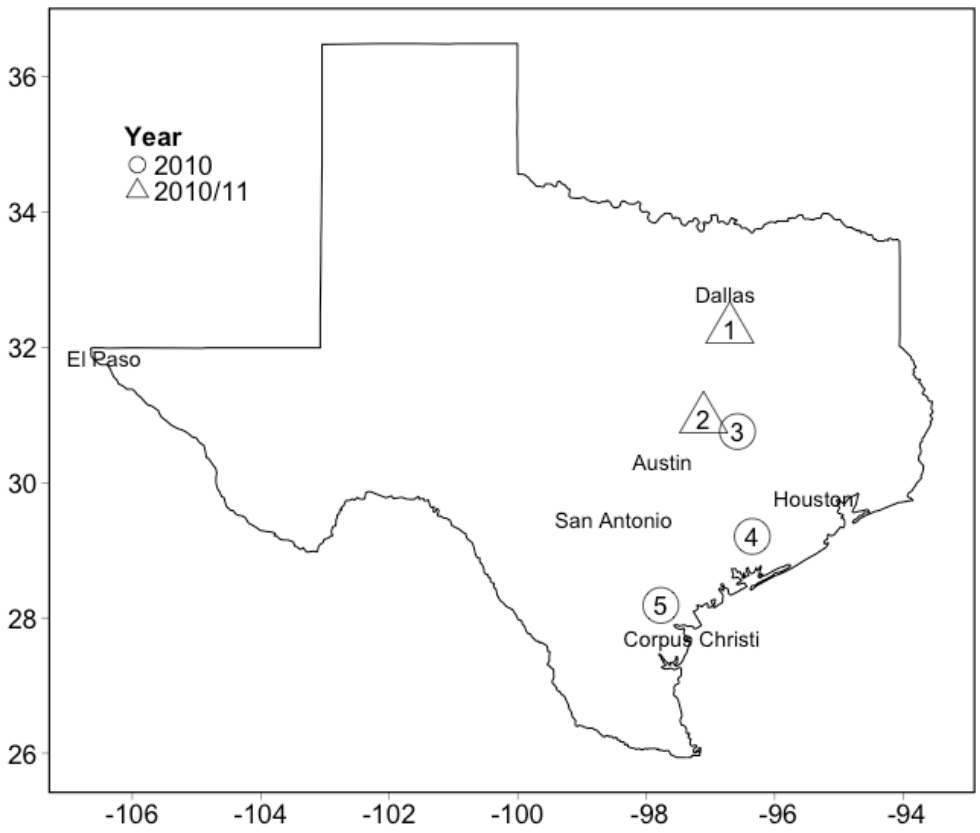


Figure 2.2. Pollinator floral visitation rate (a-b) and pollinator abundance per bowl trap (c-d) during (a,c) 2010, and (b,d) 2011.

Proximity from crop sunflowers: Far (black circles), Near (empty circles). Error bars represent 1 S.E. Significance of the proximity term in single site models are given for Far vs. Near within each site (*n* was too small for Site 4): $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ^{ns}not significant.

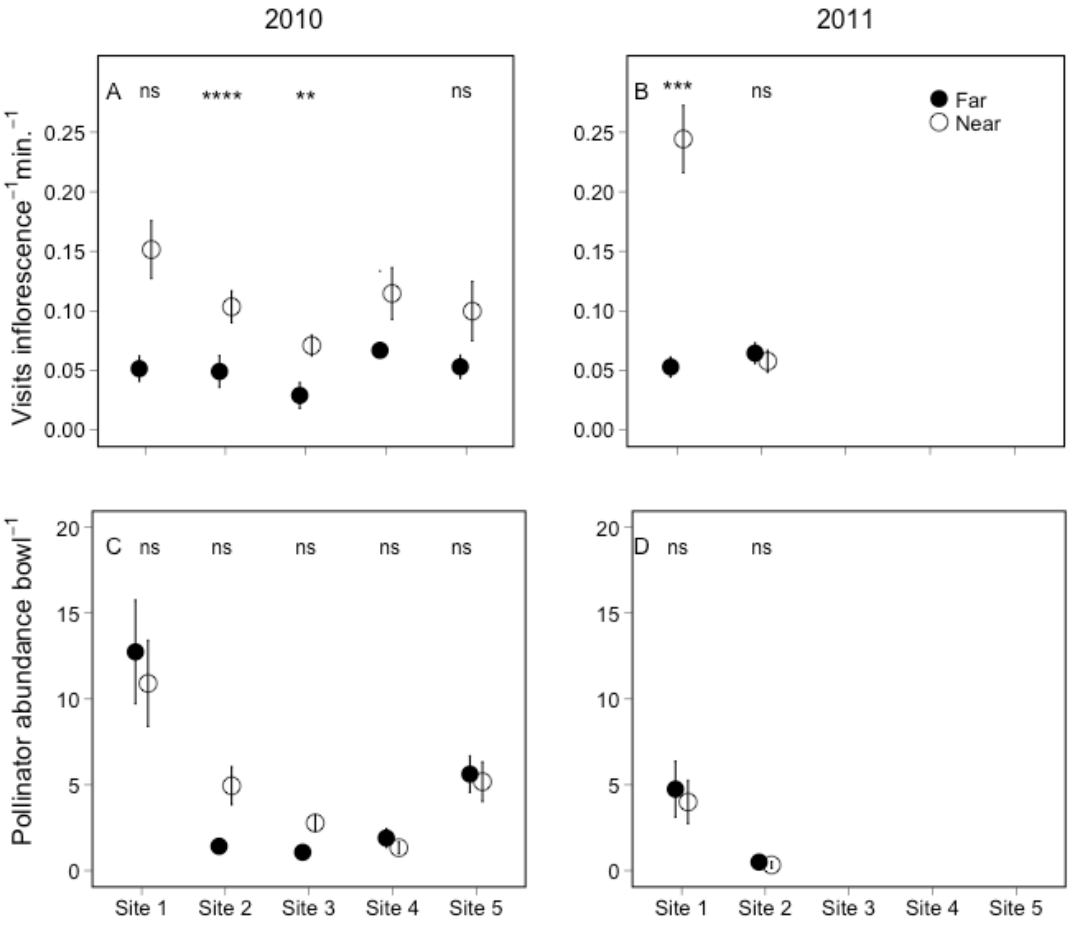


Figure 2.3. Abundance across two years (2010, 2011) for seed predators (determined via seed damage): (a,d) *Neolasioptera helianthi*; (b,e) *Isophrictis* sp.; and (c,f) *Smicronyx sordidus*.

Proximity from crop sunflowers: Far (black circles), Near (empty circles). Note that ordinates differ among panels. Error bars represent 1 S.E. Significance of the proximity term in single site models are given for Far vs. Near within each site (*n* was too small for Site 4): $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ^{ns}not significant.

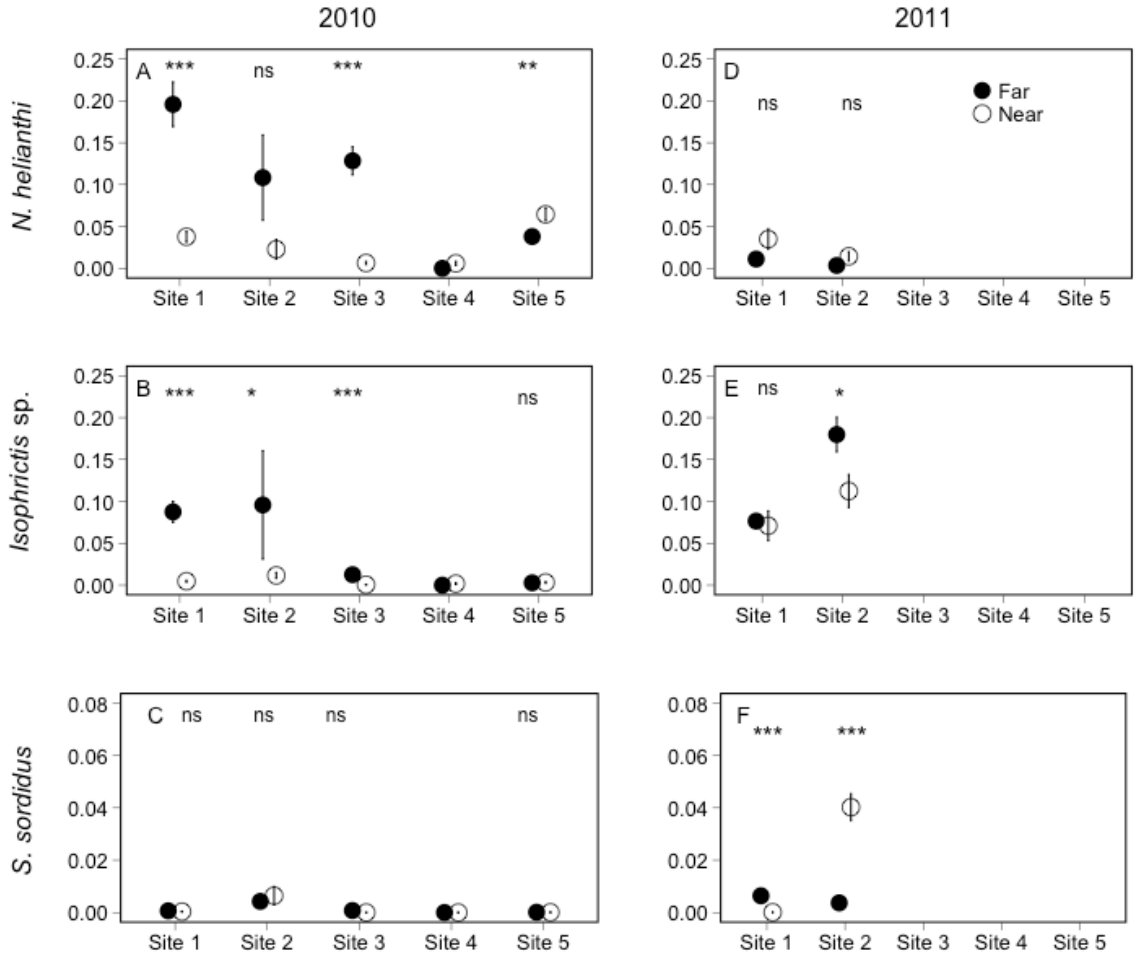


Figure 2.4. Abundance of chewing damage folivores (a) and vascular tissue damage folivores (b) in 2010.

Proximity from crop sunflowers: Far (black circles), Near (empty circles). Note that ordinates differ among panels. Error bars represent 1 S.E. Significance of planned contrasts are given for Far vs. Near within each site: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, ^{ns}not significant.

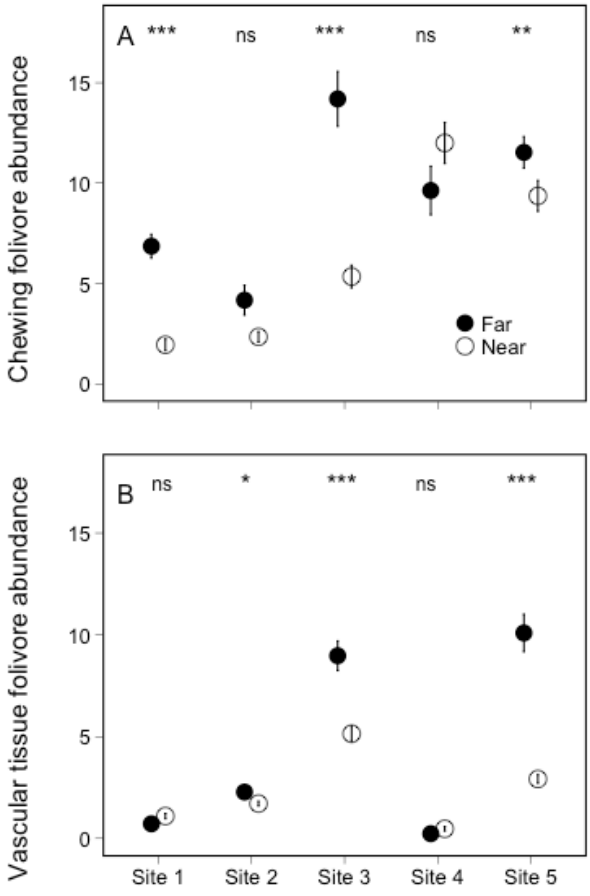
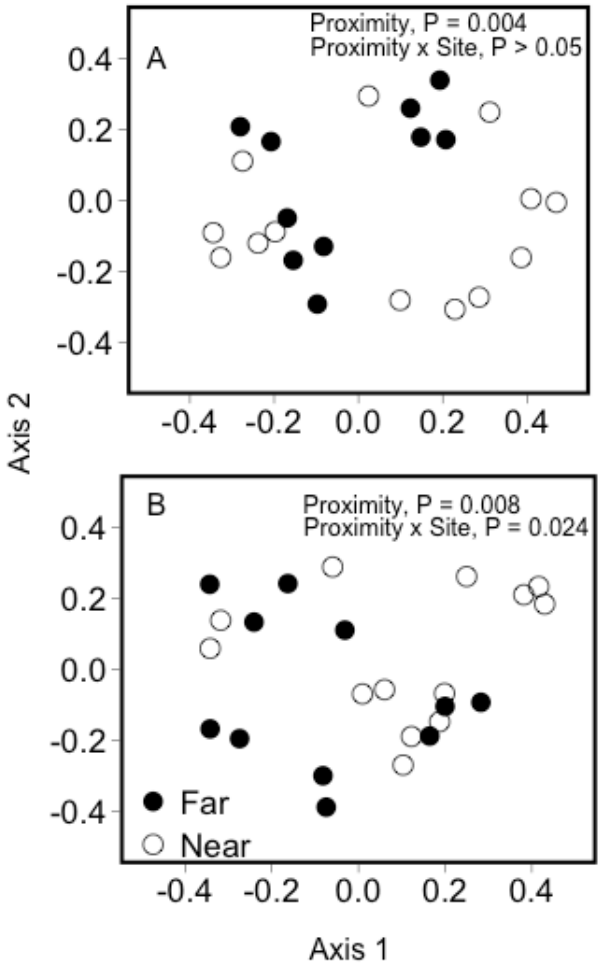


Figure 2.5. Non-metric multidimensional scaling ordination plots showing differences in community structure for (a) pollinators in 2010 and 2011 combined, (b) seed predators in 2010 and 2011 combined.

Two-dimensional stress of the ordinations: (a) 0.18 and (b) 0.07, indicating low risk for false inferences (McCune and Grace 2002).



2.11. Appendix A

Table A1. The contribution of individual species to differences among proximity treatments in pollinator community stucture in 2010 and 2011 combined (see Fig. 5a in the main paper for the NMS ordination of pollinator data). SIMPER (similarity percentages analysis) was done, and presented are percent contribution of each species to the difference between plots near versus far from sunflowers. % Contr. = percent contribution of the species to the difference between proximity treatments. % Cum. Contr. = cumulative percent contribution of the species to the difference between proximity treatments. Only the species contributing at least 1% are shown here.

Species	Taxonomy	% Contr.	% Cum. Contr.
Diadasia enavata	Hymenoptera: Apidae	21	21
Halictus ligatus	Hymenoptera: Halictidae	13	34
Apidae sp. 2	Hymenoptera: Apidae	11	45
Megachile sp. 1	Hymenoptera: Megachilidae	10	55
Megachile sp. 2	Hymenoptera: Megachilidae	6	61
Apis mellifera	Hymenoptera: Apidae	6	67
Halictidae sp. 1	Hymenoptera: Halictidae	6	73
Apidae sp. 1	Hymenoptera: Apidae	3	76
Hesperiidae sp. 1	Lepidoptera: Hesperiidae	3	79
Bombyliidae sp. 3	Diptera: Bombyliidae	3	82
Hesperiidae sp. 2	Lepidoptera: Hesperiidae	2	84
Svastra sp. 1	Hymenoptera: Apidae	2	86
Perdita sp. 2	Hymenoptera: Andrenidae	1	87
Lasioglossum sp. 1	Hymenoptera: Halictidae	1	89
Megachile sp. 3	Hymenoptera: Megachilidae	1	90
Unidentified bee 1	Hymenoptera: Apoidea (bees)	1	91
Hesperiidae sp. 3	Lepidoptera: Hesperiidae	1	92
Bombyliidae sp. 1	Diptera: Bombyliidae	1	93
Megachile sp. 4	Hymenoptera: Megachilidae	1	94
Perdita sp. 1	Hymenoptera: Andrenidae	1	95
Colias eurytheme	Lepidoptera: Pieridae	1	95
Agaostemon texanus	Hymenoptera: Halictidae	1	96
Lycinidae sp. 1	Lepidoptera: Lycinidae	1	96

Table A2. The contribution of individual seed predator species to differences among proximity treatments (Near vs. Far) in antagonist community stucture in 2010 and 2011 combined (see Fig. 5b in the main paper for the NMS ordination). SIMPER (similarity percentages analysis) was done, and presented are percent contribution of each species to the difference between Near and Far treatments. See Table A1 for further details about the analyses.

Species	Taxonomy	% Contr.	% Cum. Contr.
Isophrictis sp.	Lepidoptera: Gelechiidae	50	50
Neolasioptera helianthi	Diptera: Cecidomyiidae	46	95
Smicronyx sordidus	Coleoptera: Curculionidae	5	100

Chapter 3

3. Proximity to crop sunflowers affects patterns of natural selection in a wild relative through plant mutualists and antagonists

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To be submitted to *Evolutionary Applications* as an *Original Research Article*

3.1. Abstract

Abiotic and biotic heterogeneity result in divergent patterns of natural selection in nature. However, increasing amounts of the global terrestrial surface are homogenized by agriculture (which covers nearly 50% of terrestrial vegetated land surface) and other anthropogenic activities. Agricultural intensification leads to highly simplified biotic communities for many taxa, which may alter natural selection through biotic selective agents. In particular, crops may alter selection on traits of closely related wild relatives. We asked how crop sunflowers (*Helianthus annuus*) alter natural selection on floral traits of wild sunflowers (*H. a. texanus*). In

two years, we planted replicated paired populations of *H. a. texanus* bordering sunflower crop fields vs. approximately 2.5 km away (near natural habitat and randomly selected non-crop sunflowers). Floral traits showed significant variation in naturally occurring populations, and were highly heritable. There was significant total selection on 29% of the 225 trait/population cominations (nine floral traits across 25 wild sunflower populations). However, direct selection on floral traits was found in only 8% of cases. Total selection was more often significant far from crop sunflowers relative to near crop sunflowers. Comparing all populations in one model for 2010, direct selection on floral traits differed by proximity to crop sunflowers in 28% of cases (7.5% in 2011). Selection gradients were more heterogenous near crop sunflowers relative to far from crop sunflowers. Differences in mutualist pollinators and antagonist seed predators mediated differences in selection on flower traits near versus far from crop sunflowers. These results show that natural selection in wild plants can vary depending on the distance from natural habitat, and counterintuitively, may in some cases lead to more trait diversity in wild populations near crops vs. further away.

3.2. Introduction

In natural landscapes, abiotic and biotic heterogeneity produce spatially divergent patterns of natural selection, contributing to divergent evolutionary paths among populations. However, reduction of this natural heterogeneity, via anthropogenic alterations such as urbanization, agriculture, and introduction of invasive species, could reduce natural geographic variation in evolutionary

trajectories (Palkovacs *et al.* 2012). For example, the presence of an invasive plant and a shared herbivore altered natural selection on herbivore resistance traits in a native plant (Lau 2006); across space, joint occurrence of these species could lead to homogenized selection. Despite the fact that croplands, pastures, and rangelands covered ~50% of the global vegetated land surface as of 2005 (Foley *et al.* 2005), we don't know how agriculture alters evolution of wild plants through natural selection.

Proximity to agriculture may lead to altered evolutionary trajectories for wild species in various ways. First, crop-to-wild gene flow commonly occurs, and could effect the evolution of wild species (Ellstrand *et al.* 1999; Pilson and Prendeville 2004). One way this could happen is crop gene introgression leading to increased vigor of crop-wild hybrid plants (Campbell *et al.* 2006). This process of altered evolution through gene flow is well known, but we know much less about evolution through natural selection in agricultural mosaics. Second, drastically increased use of hebicides associated with genetically modified crops (GMO; e.g., Roundup) has lead to evolution of resistance to the herbicide in many species, e.g., two species of *Ambrosia* and three species of *Amaranthus*, both Amaranthaceae (Powles 2008). Third, fertilizer runoff from crop fields affects growth and other responses of plants along crop borders (Blackshaw *et al.* 2004; Quinn *et al.* 2007), which could drive evolution of resource-acquisition traits and competitive ability. Last, changes in abundance in, and homogenization of, biotic communities associated with agriculture (Ekroos *et al.* 2010; Chamberlain *et al.* in prep.) may affect evolution by natural selection in wild species via alteration of the presence or

abundance of selection agents. Given that many wild species now occur in human-altered landscapes, it is likely that the evolution of many species is affected by anthropogenic homogenization. However, we know little of how biotic homogenization influences evolution in wild species; no studies have experimentally examined the possible evolutionary consequences of landscape-level homogenization of biotic interactions, which requires experiments in multiple populations and a geographic perspective.

The ability of a crop to influence evolution in nearby wild plants may depend on their relatedness. This is especially true for gene flow (Ellstrand *et al.* 1999), but also for natural selection. We know that species interactions are often phylogenetically conserved, such that closely related species are likely to interact with similar species, or at least have a similar number of interactions (Gómez *et al.* 2010). Thus, in a system in which the crop and focal wild plant species are closely related, they may interact with many of the same species (e.g., share pollinators and herbivores), and furthermore may respond similarly to biotic and abiotic conditions because traits are often phylogenetically conserved (Blomberg *et al.* 2003). However, since crops have been artificially selected to be morphologically and phenologically distinct from their wild relatives, we note that traits may not always be phylogenetically conserved between crops and their wild relatives. Interactions between crops and wild relatives are especially likely when they occur in close proximity. Such situations are likely quite common; many crop plants are cultivated in locations where their wild relatives are especially abundant and diverse. Examples include sunflowers in North America, wheat

in the Middle East, corn, squash, and peppers in Mexico, and potatoes from southwestern USA to Uruguay (Jarvis *et al.* 2008).

Here, we explore how proximity of crop sunflowers (*Helianthus annuus*) to wild North American sunflowers (*Helianthus annuus* ssp*. texanus*) alters natural selection on floral traits of the latter. Specifically, we ask the following five questions: 1) Do floral traits exhibit heritable variation?; 2) How does proximity to crop sunflowers affect total and direct selection on *H.a. texanus* floral traits?; 3) Does homogeneity of selection coefficients and selection gradients on *H.a. texanus* floral traits differ with proximity to crop sunflowers?; 4) Does *H. a. texanus* pollen limitation differ with proximity to crop sunflowers?; and 5) Do mutualist pollinators and antagonist seed predators contribute differently to selection on *H. a. texanus* floral traits with proximity to crop sunflowers?

3.3. Materials and Methods

Study system

Cultivated *Helianthus annuus* and its wild congeners (sunflowers; Asteraceae) provide a highly tractable system for studying how agriculture alters the evolutionary trajectories of wild species in situations where crops and wild species share mutualists and antagonists. First, as Asteraceae have sporophytic self-incompatibility (Linder *et al.* 1998), self pollen grains do not germinate pollen tubes allowing for the quantification of outcross pollen grains deposited by pollinators. Second, in sunflower growing regions in

the US, crop and wild sunflowers can overlap for 5-6 mo. in flowering phenology (K. Whitney, pers. obs.), leading to high potential for shared pollinators (mutualists) and seed predators (antagonists) among crop and wild sunflowers. Many wild *Helianthus* species produce viable hybrid offspring with crop sunflowers (Whitton *et al.* 1997; Linder *et al.* 1998), an indication of shared insect pollinators. Third, wild *Helianthus* commonly occur along the borders of sunflower crop fields (Burke *et al.* 2002). Finally, the target wild species, *Helianthus annuus texanus*, is an annual, which is ideal for measuring lifetime fitness and selection in nature.

A diverse biotic community interacts with wild and crop sunflowers. In general, the pollinator communities of both crop and wild sunflowers are dominated by several hundred species of bees, some of which are shared between *Helianthus* species (Hurd Jr. *et al.* 1980), with honeybees particularly prevalent in crop sunflowers (Greenleaf and Kremen 2006). Many species of insect seed predators attack both wild and crop sunflowers (Charlet *et al.* 1997), and their species-specific damage to sunflower seeds is easily quantified (Whitney *et al.* 2006). Seed predator species are known to influence natural selection on sunflower traits (Whitney *et al.* 2006).

1) *Do floral traits exhibit heritable variation*?

Heritability is important because response to selection (R) is a function of selection strength (s) and heritability (H^2) (Lynch and Walsh 1998) – strongly heritable traits increase response to selection. Thus, if our analyses (below) find that natural selection on floral traits is altered by proximity to agriculture, nonzero

heritabilities for these traits would indicate that evolutionary divergence is expected as well. In a common garden, we calculated asked if floral traits varied significantly among populations, and if traits exhibited broad-sense heritability (Appendix A). However, as common garden studies cannot rule out maternal effects on phenotypic traits (Roach and Wulff 1987), we used sire-offspring regression to calculate narrow-sense heritability (Lynch and Walsh 1998). We measured sire traits and performed controlled crosses in the field in 2011 using a population from Seed source 2, then grew the offspring and measured their traits in a greenhouse in 2012, providing a lower bound for the heritability estimate. This approach is superior to measuring heritability only in the greenhouse (Riska *et al.* 1989). We maximized variation among sires by choosing sires that represented the extremes in disk diameter (large and small; a trait to which pollinators previously showed strong responses) (Hill and Thompson 1977). We bagged inflorescences on randomly chosen dams a few days before hand-pollinating them with sire inflorescences. Sires were only used in one sire x dam combination. In 2012, seeds were germinated in petri dishes, then transferred to round 1 gallon pots with Pro-Mix BX soil mix (BWI, Quakertown, PA) in the greenhouse, with no supplemental light. Plants were fertilized once with 6 g of Osmocote® fertilizer (19-6-12 NPK, Scotts-Miracle-Gro®, Marysville, OH, USA) when they were approximately 8 cm in height. Plants were watered twice per day for the first month, then watered once every two days afterwards.

We measured nine floral traits: four on the scale of inflorescences (disk diameter, ray length, ray width, number of rays, to the nearest 0.01 mm), and five on

the scale of individual disk flowers (corolla lobe size, distal throat width, distal throat length, proximal throat size, corolla tube size). Up to five individual disk flowers were collected in 70% ethanol from different inflorescences on each plant across the flowering season. We captured pictures of each individual disk flower using a Leica DFC-480 digital camera attached to a Leica DM-2500 dissecting microscope camera and Leica Application Suite (Leica Microsystems, Wetzlar, Germany), then took eight measurements (corolla lobe length, corolla lobe width, distal throat width, distal throat length, proximal throat length, proximal throat width, corolla tube length, corolla tube width; see Fig. 2) using Image J software (Rasband 2009). Using these eight measurements, we then calculated five traits for each individual disk flower as: corolla lobe size (corolla lobe length X width), distal throat width (as is), distal throat length (as is), proximal throat size (proximal throat length X width), and corolla tube size (corolla tube length X width).

We calculated narrow-sense heritability using weighted least-squares sire-offspring regression (R Development Core Team 2011) to account for variation in number of offspring per sire (mean ± 1 SE: = 4.6 ± 0.1, range = 1-12). We calculated weights using the Kempthorne-Tandon derivation from an initial regression, and then reiterated until weighted estimates of the regression coefficient converged (Lynch and Walsh 1998). Heritability was estimated as 2× the sire regression coefficient, and a lower bound for heritability was calculated following Riska et al. (1989).

Q2) How does proximity to crop sunflowers affect selection on H. a. texanus *floral traits*?

Study sites and study design

In experimental studies in 2010, we manipulated the proximity of *H. a. texanus* to crop sunflowers by transplanting arrays of 80-100 seedlings either near crop sunflowers (plot of *H. a. texanus* 10 m from the crop) or far from them (plot 2.5 km from any sunflower crop, near a different crop bordering natural habitat [e.g., tree lines] whenever possible). Plots were replicated at each of five farms in TX (Fig 1), with two near and two far plots per farm. The crop sunflowers planted by farmers were all Clearfield® variety, which are not genetically modified, but have been artificially selected to be resistant to the imidazolinone herbicides (Sala *et al.* 2008), which was sprayed on the crop sunflowers to reduce weeds. All the crops in my study were grown for sunflower oil production. The proximity treatment was crossed factorially with a seed origin treatment (seeds from one of two wild populations collected in 2009) to enhance the generality of results. In 2011, we used the same design as 2010 (proximity treatment crossed with seed origin treatment), but only used two of the five sites used in 2010 (Sites 1 and 2; see Fig. 1). In 2010, one plot at Site 1 was lost in 2010 due to accidental herbicide spraying, and two plots were lost at Site 4 due to flooding. In 2011, an extreme drought caused wild pigs to seek out wet roots early in the season damaged two plots at Site 2, and then plants were replaced with new seedlings.

Seedlings were obtained by nicking seeds with a razor blade, and germinating them on damp filter paper in late February each year (2010 and 2011). Germinating

seeds were kept in the absence of light at room temperature, and were moved into the light after they produced fine root hairs. Seeds were kept damp at all times during germination. Approximately eight-day-old seedlings were transplanted into peat pellets (J30100 Super; Jiffy, Denmark) and were grown in a Rice University greenhouse for approximately four weeks before transplanting to the field in approximately early- to mid-April. Plants were watered in the field every three to five days by hand until the end of May.

We quantified fitness as whole-plant seed production for each plant. We used mesh bags (8 cm x 8 cm, made from plastic mesh; DelStar Technologies, Delaware) to capture seeds from three to six inflorescences per plant (following methods of Whitney *et al.* 2006). At the end of the season (September), after seeds had matured and plants had senesced, we counted the total number of inflorescences per plant and collected bagged inflorescences. Mean seed production per inflorescence was counted, and multiplied by inflorescence number to estimate whole plant seed production. *H.a. annuus* is an annual, so this measure is a lifetime fitness measure. To account for possible scaling of seed production and flower traits with plant size, we calculated plant volume at the end of the season by measuring height to the tallest inflorescence (to the nearest cm) and diameter of the stem at the base (to the nearest 0.1 mm) (Whitney *et al.* 2006). Plant volume was calculated as a $\pi r^2 h$, where *r* is the radius of the stem at the base, and *h* is the height. We recorded plant abundance by counting the number of plants that survived to reproduction. We measured four inflorescence-level and five disk flower- level traits for each plant following methods above.

Phenotypic selection analyses

For each plot, we performed phenotypic selection analysis following Lande and Arnold (1983). Relative fitness (calculated within each plot) was used in the analyses. As predictor variables, we included four inflorescence traits (disk diameter, ray length, ray width, number of rays), and five disk floral traits (corolla lobe size, distal throat width, distal throat length, proximal throat size, corolla tube size). We also included plant volume in each model to account for indirect selection on floral traits via direct selection on plant size. All traits were transformed as necessary to improve normality and then standardized (mean = 0, sd = 1). All variance inflation factors were < 5.0 and all condition indices were < 6.0. Selection gradients (β) were calculated as the partial regression coefficients simultaneously fitted to all traits in a single multiple regression analysis. Linear selection differentials (s') were calculated as the covariance between each trait and relative fitness; significance of differentials was assessed through the *P*-value of Pearson correlation tests of each trait on relative fitness. Due to limited number of plants in each population ($\bar{x} \pm 1\ s.e.$; 90.5 ± 4.9, n = 26 plots), we were unable to estimate nonlinear selection gradients or differentials, or test for correlational selection using trait X trait interactions. We calculated Pearson correlations among floral traits for each population, and report the average correlation for each pairwise trait combination (see Appendix Table B2).

ANCOVA was used to assess whether populations experienced different selective pressures near versus far from crop sunflowers, and whether selection

varied among sites or years. The response variable was relative fitness. We ran a model for 2010, and a second model for 2010/2011 (which included only sites 1 and 2 which were replicated in both years). For total selection, ANCOVA models were run for each trait separately for 2010 and 2010/2011 combined for Sites 1 and 2. Models included the fixed factors site, proximity to sunflower crop, and their interactions with each other the single trait. Population nested within site, seed source, and proximity was included as a random effect. The multi-year model was similar to the 2010 model, but included year as an additional factor. For direct selection, the 2010 model included the model above for total selection, but included all nine floral traits. In preliminary analyses, seed source was not significant, and so was excluded in the final models. A significant interaction between a trait and proximity, trait × site × proximity, trait × year × proximity, or trait × site × proximity × year would indicate that total or direct selection on floral traits varied with proximity to crop sunflowers. Analyses were done with PROC MIXED in SAS (SAS 2003).

Q3) *Does homogeneity of selection coefficients and selection gradients for* H. a. texanus *floral traits differ with proximity to crop sunflowers*?

We hypothesized that variation in selective regimes would differ near vs. far from crop sunflowers. Using selection gradients (β) and selection differentials (s') calculated in the above analyses on individual populations, we compared variances using *F*-tests ($F = s^2_{far}/s^2_{near}$). Significantly reduced (or increased) variance of selection differentials or gradients in near relative to the far populations would suggest that

natural selection is more (or less) homogenous in closer proximity to the crop species, and would indicate that agriculture causes large-scale spatial alteration of the patterns of natural selection.

Q4) *Does* H. a. texanus *pollen limitation differ with proximity to crop sunflowers*?

We conducted an experiment to test the hypothesis that pollen limitation is greater far from sunflower crops relative to near them. This should result if our hypothesis is true that pollinators should be more abundant near sunflower crops; greater pollinator visitation should lead to decreased pollen limitation (Knight *et al.* 2005). We randomly selected 40 plants in each of two plots at Site 2, one plot near sunflower crops, and one plot far from sunflower crops. We randomly allocated 20 plants in each plot to the experimental hand-pollination treatment, and 20 to the control, open-pollination treatment. All flowering inflorescences of plants in the hand-pollinated treatment received excess pollen from an entire inflorescence from plants selected at random adjacent to the plot. Control, open-pollinated, plants received no extra pollen other than that from pollinator visitation. We measured whole plant fitness on each plant as average seeds per inflorescence (~ 6 inflorescences per plant) X number of inflorescences per plant.

We examined if plant fitness was pollen-limited overall and if the extent of pollen-limitation differed by proximity to sunflower crops using ANOVA. The response variable was whole plant fitness, while the explanatory variables were pollination treatment (hand- vs. open-pollination), proximity to sunflower crops (near vs. far), and

their interaction. If the interaction is significant this would suggest that pollen limitation differs by proximity to sunflower crops. We excluded one plant as an extreme outlier. We also determined if plant size (using plant volume) differences could account for differences in plant fitness using ANOVA, separately for each plot.

Q5) *Do mutualist pollinators and antagonist seed predators contribute differently to selection on* H. a. texanus *floral traits with proximity to crop sunflowers*?

We used multi-group structural equation models (SEM) to compare the contribution of mutualists versus antagonists to selection on floral traits near versus far from crop sunflowers (Fig. 5). Although SEM by itself cannot determine causation, there is evidence from nature that some causal pathways in our models are valid. For example, hand-pollination studies have shown that plants are often pollen limited, showing that pollinators greatly affect plant fitness (Knight *et al.* 2005). Seed predators have negative effects on plant fitness in *H. a. texanus* (Whitney *et al.* 2006).

Seed predators

We estimated seed predator damage on all plants in each sunflower plot by placing net bags on three to six inflorescences per plant after pollination, but before seed drop occurred, to allow ample time for seed predators to interact with the inflorescence. We collected bagged inflorescences at the end of the season, after seeds in inflorescences had matured, and plants had senesced. We pooled all inflorescences, and then sub-

sampled ca. 80 seeds with x10 dissecting microscope to quantify species-specific damage for *Neolasioptera helianthi* (Diptera: Cecidomyiidae), *Isophrictis* sp. (Lepidoptera: Gelechiidae), and *Smicronyx sordidus* (Coleoptera: Curculionidae). Total numbers of seeds damaged per plant were extrapolated using counts of total inflorescences per plant.

Pollen deposition

As pollen deposition is often correlated with pollinator visitation rate (Engel and Irwin 2003), pollen deposition was measured as an estimate of pollinator visitation on the individual plant level, allowing us to connect pollinator behavior to selection on floral traits. We collected XXX stigmas in the field from up to eight inflorescences per plant during the season. The stigmas were squashed under a microscope slide in glycerin, photographed with flourescence microscopy (SCOPE DETAILS), and pollen grains counted with a macro program written by SAC for Image J (Rasband 2009). We estimated pollen deposition per plant (average no. pollen grains/stigma X 100 flowers/inflorescence x no. inflorescences). We assume a constant number of flowers per inflorescence (100) as we do not have data on variation in this trait.

Data analysis

We conducted path analysis to test the relative strengths of mutualist- versus antagonist-mediated selection on wild sunflower floral traits across two contexts (near

versus far from crop sunflowers). We constructed a plausible a priori model that links floral traits to pollinator deposition and seed predators to plant fitness (Fig. 5). Variables included in the model were inflorescence traits, disk flower traits, *Isophrictis* sp. abundance, *N. helianthi* abundance, pollinator visitation, plant volume, and relative fitness (*Smicronyx sordidus* were excluded due to very low abundance). As there were relatively few plants per population and nine floral traits, we created just two variables for floral traits (one for inflorescence traits and one for disk flower traits) by extracting the first principal component from two separate principal components analyses (PCAs) using the *vegan* package in R (R Development Core Team 2011). All traits were positively correlated with the first principal component; thus, positive coefficients in the paths connecting these composite variables to fitness would represent positive selection on the traits. All traits were standardized ($\bar{x}$ = 0, s.d. = 1) prior to analysis, and traits were log-transformed as needed to improve normality; whole plant seed production was relativized to the mean of the population. We conducted multi-group analysis on near-far pairs of populations for which we had adequate data: Sites 1 and 2 in 2011 [I will soon have data on more sites]. For each site, we compared model fit for a fully constrained (all paths equivalent between treatments) model to a model with no constraints, and if fit was significantly better without all paths constrained we iteratively modified (removed or added paths) each model based on model diagnostics, standardized residuals and modification indices (Arbuckle 2003). Then we tested if individual path coefficients differed between treatments (near versus far from crop sunflowers) using the critical ratio, the difference between two parameters divided by

the standard error of the difference. The critical ratio is compared with the standard normal distribution to determine significance at α = 0.05 (Arbuckle 2003). The units of observation were individual plants. We report standardized path coefficients. Analyses used Amos v.20 (Arbuckle 2003).

3.4. Results

Q1) *Do floral traits exhibit heritable variation*?

Narrow-sense heritabilities ranged from 0.15 to 0.64 (Table 1). Five traits displayed heritability values different from zero: disk diameter, ray length, number of rays, distal throat length, and distal throat width. However, four traits showed heritability values not different from zero: ray width, corolla lobe size, proximal throat size, and corolla tube size. These results suggest substantial potential for many *H. a. texanus* floral traits to change in response to natural selection.

Q2) *How does proximity to crop sunflowers affect selection on* H. a. texanus *floral traits*?

Selection across all populations - total selection. In 2010, total selection on floral traits differed by proximity to crop sunflowers in 22% of possible cases (four of 18 significant trait × proximity, or trait × proximity × site in ANCOVA). Total selection on disk diameter was significantly different near versus far from crop sunflowers at some sites but not others (P = 0.002; Table B2). For example, there was total selection for larger disk diameter at Site 2 in all four populations, but at

the other four sites total selection for larger disk diameter was only found in some populations. Like disk diameter, total selection on increased ray length differed near versus far from crop sunflowers only at some sites ($P = 0.016$; Table B2). Total selection on number of rays was significantly different near versus far from crop sunflowers at some sites but not others ($P = 0.001$; Table B2). Total selection on proximal throat size was significantly different near versus far from crop sunflowers at some sites but not others ($P = 0.009$; Table B2).

In an analysis including 2010 and 2011 data for Sites 1 and 2, we asked whether total selection on floral traits differed by proximity to crop sunflowers depended on the year. Whether total selection differed by proximity to crop sunflowers on average across all sites depended on the year (trait x year x proximity) in three traits: disk diameter, ray length, and ray width (Table B2). In addition, whether total selection differed by proximity to crop sunflowers depended on the year and site (trait x year x site x proximity) in six traits: disk diameter, ray length, ray width, number of rays, corolla lobe size, and proximal throat size (Table B2). For example, there was marginally significant total selection for larger disk diameter at one of the near populations at Site 1 in 2010 ($\beta = 0.04$, $P < 0.06$; Table B5), but there was significant positive selection at a far population in 2011 at the same site ($\beta = 0.14$, $P < 0.05$; Table B5). In addition, there was significant positive selection on disk diameter at one of the far populations at Site 2 in 2010 ($\beta = 0.05$, $P < 0.05$; Table B5), but no significant selection on the trait at any populations at the same site in 2011.

Selection across all populations - direct selection. In 2010, direct selection on floral traits differed by proximity to crop sunflowers in 11% of possible cases (two of 18 significant trait × proximity, or trait × proximity × site in ANCOVA). Direct selection on disk diameter was significantly different near versus far from crop sunflowers at some sites but not others ($P = 0.025$; Table B3). For example, there was direct selection for larger disk diameter at one of the far populations, but not at the near populations at Site 2; while there was direct selection for larger disk diameter at one of the near populations, but not at the far populations at Site 3 (Table B3). Selection on ray length was significantly different overall near relative to far from crop sunflowers ($F = 5.29$, $P = 0.022$; Table B3). Selection on distal throat length was significant overall ($F = 4.74$, $P = 0.030$; Table B3), but did not differ among sites or by proximity. Selection on corolla lobe size was significantly different among sites ($F = 3.10$, $P = 0.026$; Table B3), but did not differ near relative to far from crop sunflowers. Selection on number of rays, distal throat width, proximal throat size, or floral tube size did not significantly differ among sites or by proximity (Table B3).

In an analysis including 2010 and 2011 data for Sites 1 and 2, we asked whether direct selection on floral traits differed by proximity to crop sunflowers depended on the year. Whether direct selection differed by proximity to crops depended on the year in two traits (ANCOVA: trait x proximity x site x year; Fig. 3, Table B4): disk diameter ($F = 5.01$, $P = 0.007$) and corolla lobe size ($F = 3.35$, $P = 0.036$). There was marginally significant selection for larger disk diameter at one of the near populations at Site 1 in 2010 ($\beta = 0.04$, $P < 0.06$; Table B4), but there was

significant positive selection at a far population in 2011 at the same site (β = 0.14, $P < 0.05$; Table B4). In addition, there was significant positive selection on disk diameter at one of the far populations at Site 2 in 2010 (β = 0.05, $P < 0.05$; Table B4), but no significant selection on the trait at any populations at the same site in 2011. For corolla lobe size, there was significant negative selection on the trait at one of the near populations at Site 2 in 2011 (β = -0.12, $P < 0.01$; Table B6), but no significant selection on the trait in any other populations in any sites or years (Table B6). Direct selection on ray length, ray width, number of rays, distal throat length, distal throat width, proximal throat size, or floral tube size did not significantly differ among years (Table B4).

Analyses within each population. Overall, there was significant total selection (*s*') in 29% of the cases measured (65 out of 225 combinations of nine floral traits × 25 populations; Table B5; Fig. 3). Total selection was significant more often in inflorescence-level traits (52%, 55 out of 100) than in disk flower traits (10%, 13 out of 125). There was significant total selection in a large percentage of populations in inflorescence level traits, e.g. for increased disk diameter (56% of populations), increased number of rays (52%), increased ray length (60%), and increased ray width (40%; one population experienced selection for decreased ray width; Table B4). However, many fewer populations experienced significant total selection for disk flower traits, e.g. for increased corolla lobe size (8% of populations), increased distal throat width (0%), increased distal throat length (12%), increased proximal throat size (16%), and increased corolla tube size (12%).

Selection gradients (β) revealed that some of the total selection was due to selection on correlated characters. Significant direct selection was found in only 7% of the cases measured (13 out of 186; Table B6; Fig. 3). Like total selection, direct selection was significant more often in inflorescence level traits (8% or 9 out of 96 cases) than in individual flower level traits (6% or 5 out of 90 cases). There was significant direct selection in a small percentage of populations in inflorescence level traits, e.g. for increased disk diameter (13% of populations), increased number of rays (13%), increased ray length (4%), and increased ray width (4%). Very few populations experienced significant direct selection on individual flower traits, e.g. for decreased corolla lobe size (6% of populations), decreased distal throat width (11%), decreased distal throat length (0%), decreased proximal throat size (6%), and decreased corolla tube size (6%).

Q3) *Does homogeneity of selection coefficients and selection gradients for* H. a. texanus *floral traits differ with proximity to crop sunflowers*?

Selection on floral traits of wild sunflowers was more heterogeneous near crop sunflowers relative to far from crop sunflowers. While variance of selection differentials (total selection on a trait) did not differ near versus far from crop sunflowers (*F*-test for homogeneity of variances, with $H_o = 1$; ratio of variances [Far/Near] = 1.18, $F_{98,125} = 1.16$, $P = 0.422$), variance of selection gradients (selection accounting for correlations with other traits) was greater near crop sunflowers relative to far from crop sunflowers (ratio of variances [Far/Near] = 0.41, $F_{83,101} = 0.53$, $P = 0.003$; Fig. 4).

Q4) *Does* H. a. texanus *pollen limitation differ with proximity to crop sunflowers*?

There was no pollen limitation either near or far from sunflower crops (ANOVA; $F_{1,74}$ = 0.38, P = 0.542). In addition, plant fitness did not differ near relative to far from sunflower crops ($F_{1,74}$ = 0.16, P = 0.688), and there was no interaction between proximity to sunflower crops and the hand-pollination treatment ($F_{1,74}$ = 2.26, P = 0.137). Plants did not differ in volume in the far plot (ANOVA; $F_{1,35}$ = 0.05, P = 0.821) or near plot (ANOVA; $F_{1,38}$ = 0.32, P = 0.575), suggesting that differences in plant size could not account for any differences in fitness between hand- and open-pollination treatments.

5) *Does selection mediated by mutualists and antagonists differ with proximity to crop sunflowers*?

At Site 1 in 2011, the path from pollen deposition to relative fitness was significantly different far versus near from sunflower crops (Fig. 5 A,B;). Pollinator visitation had a much larger effect on fitness in the far population (β = 0.67) than the near population (β = 0.36). Pollinators (via pollen deposition) mediated stronger direct selection on floral traits far (β = -0.07) relative to near (β = -0.02) crop sunflowers, and stronger direct selection on inflorescence traits far (β = -0.06) relative to near (β = 0.004) crop sunflowers. In addition, the relationship between floral traits and *Isophrictis* sp. differed significantly far versus near crop sunflowers (Fig. 5 A,B). *Isophrictis* sp. abundance was negatively related to floral traits in the far population (β = -0.19), while

the relationship was positive in the near population (β = 0.18). Likewise, *Isophrictis* sp. abundance was negatively related to inflorescence traits in the far population (β = -0.16), while the relationship was positive in the near population (β = 0.15). *Isophrictis* sp. mediated stronger direct selection on floral traits far (β = 0.02) relative to near (β = -0.01) crop sunflowers, and stronger direct selection on inflorescence traits far (β = 0.02) relative to near (β = -0.01) crop sunflowers.

At Site 2 in 2011, the path from *Isophrictis* sp. abundance to relative fitness differed near versus far from crop sunflowers (Fig. 5 C,D). *Isophrictis* sp. abundance had a larger negative impact on plant fitness far from crops (β = -0.22) relative to near them (β = -0.04). Therefore, *Isophrictis* sp. mediated stronger selection on floral traits near crops (β = -0.01) relative to far from them (β = 0.002), and mediated stronger selection on inflorescence traits near crops (β = 0.02) relative to far from them (β = -0.01). Like Site 1 in 2011, pollinators (via pollen deposition) mediated stronger direct selection on floral traits far (β = -0.06) relative to near (β = -0.03) crop sunflowers, but not for inflorescence traits, where there was stronger direct selection on inflorescence traits near (β = 0.01) relative to far from (β = -0.004) crop sunflowers.

3.5. Discussion

Global terrestrial land use is dominated by agriculture, which creates homogenized biotic and abiotic environments. However, we know little about how this land use type influences evolution by natural selection in plants that occur in

agricultural landscapes. We planted experimental populations of a wild annual sunflower, *H. a. texanus*, either bordering crop sunflowers (*H. annuus*) or bordering other crops across five sites in Texas. We showed that natural selection on heritable floral traits often differed near versus far from crop sunflowers. In addition, selection was more variable among populations of *H. a. texanus* near crop sunflowers relative to populations far from crop sunflowers. Counterintuitively, this suggests that natural selection can be heterogeneous in agricultural landscapes, even next to the same crop. The fact that selection was more heterogeneous next to the same crop, instead of near a natural habitat suggests that diverse natural selection environments can occur in often biotically homogenized landscapes. Last, we showed that differences in mutualist pollinators and antagonist seed predators were associated with differences in selection near versus far from crop sunflowers.

How does proximity to crop sunflowers affect selection on H. a. texanus *floral traits*?

We found that natural selection on floral traits was affected by proximity to crop sunflowers. Selection on two individual floral traits differed near versus far from crop sunflowers in 2010. This could have important consequences for the evolution of wild, as well as weed plants in agricultural landscapes. Many wild plant species are labeled as ruderal species, in which agricultural landscapes may be their primary habitat. Thus, altered selection due to crop proximity could be an essential part of the evolutionary dynamics for ruderal plant species since much of their range may include agriculture.

Our results suggest that in general wild plants will experience different selection on phenotypic traits in or near natural habitat versus near crops. Crops that are closely related to wild plants should have a greater effect on selection on traits of the wild plant. Weeds are economically important plants because they cause significant crop yield losses (Sinden *et al.* 2004). Attention is paid to understanding the physiological and molecular basis of resistance in weeds, yet few studies have focused on the evolutionary processes themselves (Neve *et al.* 2009). Understanding natural selection on traits that influence weediness will benefit research on weed control.

Does homogeneity of selection coefficients and selection gradients on H. a. texanus *floral traits differ with proximity to crop sunflowers*?

We found significant spatial variation in selection on *H. a. texanus* floral traits. In particular, selection on *H. a. texanus* floral traits was more heterogeneous near crop sunflowers relative to near other crops. In addition, selection for some floral traits varied among sites. This finding was contrary to our expectation that natural selection would be homogenized in *H. a. texanus* populations near crop sunflowers. This suggests that *H. a. texanus* populations in agricultural landscapes may experience more diverse selective trajectories near their crop relatives, which increases the potential for increased *H. a. texanus* trait diversity near crop sunflowers. Across a landscape, wild plants experience a different selective environment near their crop relatives versus near other crops. In a similar way, in natural landscapes, natural selection was stronger for focal plants growing near closely related conspecific neighbors (Donohue 2003), and we

predict that selection is likely stronger in focal species when nearby species are closely related when species level traits and species interactions are phylogenetically conserved (Gómez *et al.* 2010).

Implications for the geographic mosaic theory of coevolution

Spatial variation in selection in agricultural landscapes may add new complexity to the geographic mosaic theory (GMT; Thompson 2005). The GMT posits that there is geographic variation in natural selection, reciprocal selection only happens in some locations, and genetic structure constantly changes to alter geographically variable selection. Previous research on the GMT has focused on relatively pristine landscapes. However, highly modified agricultural landscapes relative to pristine landscapes may have different evolutionary consequences. As John Thompson (p. 355; 2005) remarked with respect to agriculture, "We can guess that these human-produced mosaics have essentially the same effects on...coevolutionary dynamics as those imposed by natural mosaics, but we simply do not know". In some ways our results confirm that natural selection in agricultural landscapes is similar to natural landscapes. For example, in this study populations of *H. a. texanus* at the same site differed in selection outcomes near versus far from crop sunflowers over a distance of only ~2.5 km (Table B2, B3), patterns seen at similarly small scales in natural landscapes (Craig *et al.* 2007; Gómez *et al.* 2009; Smith *et al.* 2011). However, in other ways our results suggest a result different from that seen in pristine landscapes. That is, we saw more heterogeneous selection on floral traits near the same homogenous crop (sunflowers) relative to far from the crop near

natural habitat. This is likely due to greater biodiversity of pollinators associated with crop sunflowers, which varies spatially, leading to variable selection in wild sunflowers. Furthermore, variation in plant fitness within a population, common in empirical systems and fundamental to the GMT (Thompson 2005), are less likely in highly simplified agricultural landscapes. This could contribute to weaker selection in agricultural landscapes.

Mechanisms for differential selection by proximity to crops

There are a number of mechanisms that could drive variable selection due to proximity to crops in wild plants. We examined one likely mechanism in our system: alteration of potential biotic agents of selection. Differences in biotic agents near versus far from crop sunflowers could drive differences in selection on plant traits. We have shown changes in abundance and community structure of mutualist (pollinators) and antagonist (seed predators) putative agents of selection on floral traits in *H. a. texanus* due to crop sunflower proximity (Chamberlain *et al*. in prep). Here, we showed that changes in these mutualists and antagonists mediated differences in selection on floral traits at two sites (Fig. 5). For example, at Site 1 mutualist pollinators were stronger mediators of selection on floral traits far from crop sunflowers (Fig 5A,B), while antagonist seed predator *Isophrictis* sp. mediated positive selection on floral traits far from crop sunflowers, but negative selection near them.

Mutualists and antagonists are differentially influenced by crop management. For example, in a meta-analysis of responses of insects to agriculture, antagonists

increased more in abundance than did mutualists in response to decreasing agricultural intensity (Chamberlain, unpublished analysis based on data from Batary *et al.* 2011). Factors that could be important in this result are chemical use (herbicides, pesticides, fertilizers), soil treatment (tilling vs. no-till), and crop rotation (Thrall *et al.* 2011). Pesticides and herbicides could have played a role in our system in making arthropods emigrate out of crop fields, possibly to interact with weed or wild plants along crop borders. However, management in our fields was relatively similar because of the same sunflower crop variety in all locations. Although, perhaps large-scale agriculture, as examined in this study, is less likely than smaller scale agriculture embedded within natural habitat to experience flows across borders given the very intensive and extensive use of land with little non-crop habitat (Blitzer *et al.* 2012).

Caveats

We acknowledge a few caveats to our study. First, our results may not generalize to systems where a focal wild species grows near an unrelated crop. Crops and unrelated wild plants are less likely to share species interactions as species interactions are phylogenetically conserved (Gómez *et al.* 2010). We planted wild sunflowers next to other crops at random in this study, but future studies could manipulate relatedness of the crop to examine whether selection effects differ with crop type. In addition, although floral traits in this study experienced selection, different classes of traits may experience different selective consequences due to different agricultural factors. For example, traits related to nutrient acquisition and competition, such as root biomass

and growth rate, are likely to experience natural selection due to crop fertilizer runoff and tilling. Floral traits are less likely to respond to these crop management factors, and thus may represent a conservative test for the presence of crop proximity effects on natural selection. Third, individual fields where wild plants occur are next to different crops each year or even within years. Because of this crop rotation, the effect of proximity to crops on natural selection on wild plant traits may be lessened. Last, *H. a. texanus* is largely a ruderal species, so is not found in pristine habitats with which conservation is concerned. Natural selection on traits in wild species in pristine habitats is likely to be more heterogeneous relative to biotically simplified agricultural habitats. Future studies that make this comparison will add significantly to our understanding of the evolutionary consequences of agricultural habitats.

3.6. Conclusion

We show that natural selection on floral traits in a wild plant species (*Helianthus annuus texanus*) was significantly altered by proximity to its crop relative (sunflowers, *H. annuus*). Importantly, selection on floral traits in populations of *H. a. texanus* near their sunflower crop relatives was more heterogeneous compared to populations of *H. a. texanus* near other crops. Furthermore, changes in abundance and community composition of mutualist pollinators and antagonist seed predators mediated differences in selection on floral traits. These results suggest that, despite the common finding that biotic communities are homogenized in agricultural landscapes, there are

complex patterns of natural selection in agricultural landscapes, partly mediated by mutualists and antagonists.

3.7. Acknowledgements

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3.9. Tables

Table 3.1. Results of narrow-sense heritability experiments for four inflorescence, and five floral traits.

Narrow-sense values were estimated using sire-offspring regression with sire traits measured in the field, and offspring traits measured in a greenhouse (see Methods section for details). Tests are for heritability estimates significantly different from zero. See Fig. 2 for diagram of floral traits.

	Narrow-sense	
Trait	h^2	95% CI
Inflorescence traits		
Disk diameter (DD)	0.47	(0.318, 0.614)
Ray length (RL)	0.28	(0.001, 0.560)
Ray width (RW)	0.25	(-0.103, 0.602)
No. rays (NR)	0.42	(0.162, 0.681)
Floral traits		
Distal throat length (DTL)	0.39	(0.088, 0.696)
Distal throat width (DTW)	0.64	(0.326, 0.955)
Corolla lobe size (CS)	0.28	(-0.018, 0.573)
Proximal throat size (PTS)	0.20	(-0.077, 0.474)
Corolla tube size (TS)	0.15	(-0.161, 0.458)

3.10. Figures

Figure 3.1 Map of natural populations from which seeds were collected in 2005 (population E), 2006 (population D), and 2009 (populations A-C, F-I) and where experimental studies were conducted in 2010 and 2011 (Sites 1-5).

Note that the five sites (1-5) were used in 2010, of which two sites (1 and 2) were also used in 2011.

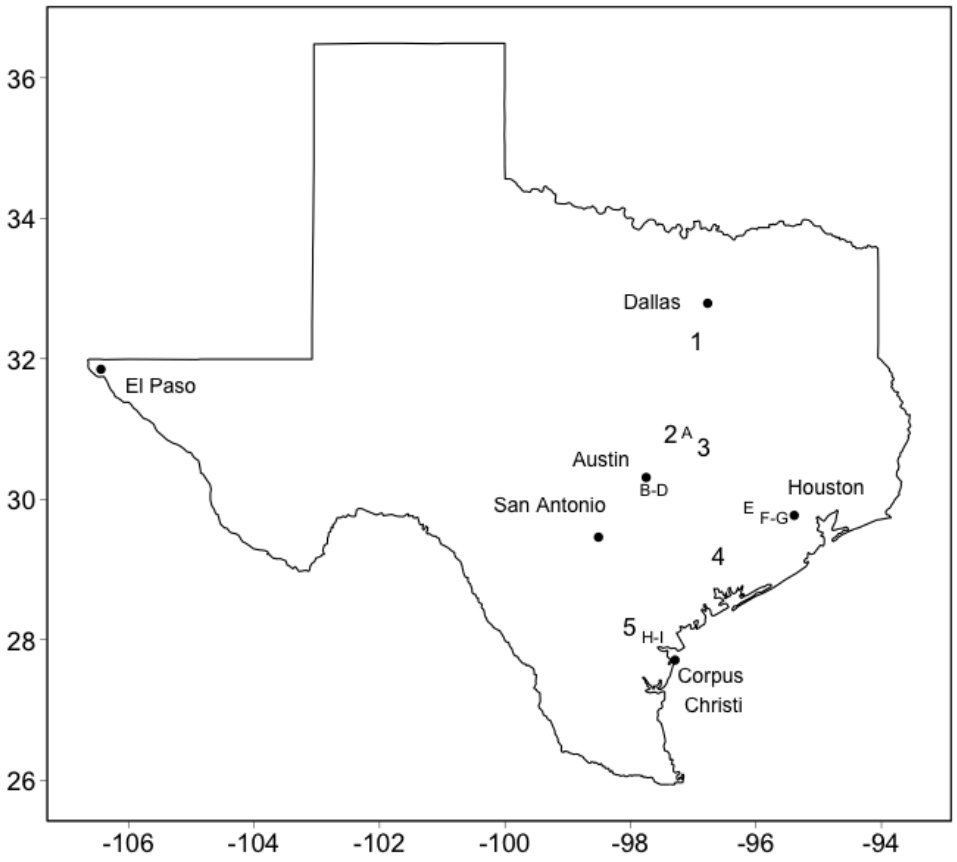


Figure 3.2. Diagram representing traits measured on individual disk flowers in *H.a. texanus*.

CW: corolla lobe width; CL: corolla lobe length; DTW: distal throat width; DTL: distal throat length; PTL: proximal throat length; PTW: proximal throat width; TL: corolla tube length; TW: corolla tube width. [**a photograph of a disk flower will be included with the diagram, but not included for now to decrease file size**]

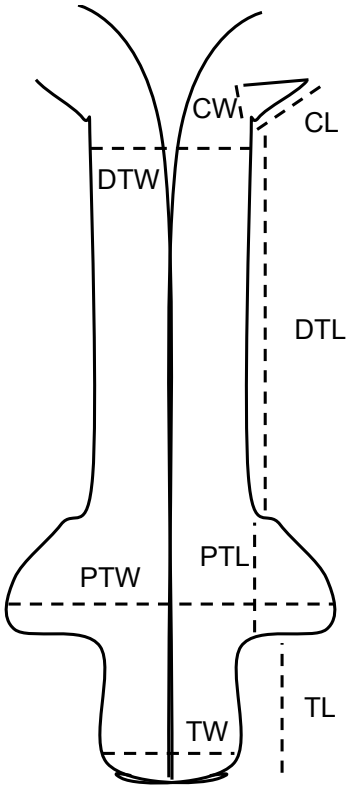


Figure 3.3. Mean (± 1 *s.e.*) magnitude of selection differentials and selection gradients for all populations far (fille circle) and near (empty circle) from crop sunflowers.

Values were calculated independently for each population (see Methods section for details), and then mean values calculated across population values. There are no significance statistics associated with these values calculated within populations, but see Q2 in Results. See Fig. 2 caption for trait abbreviations.

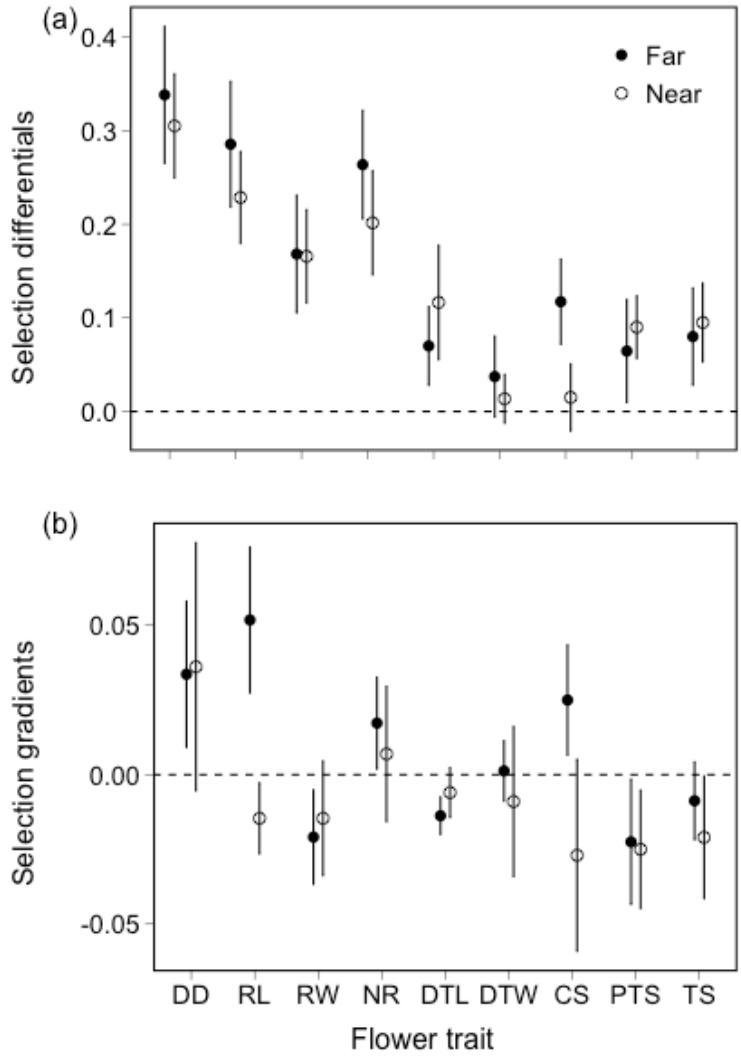


Figure 3.4. Standardized selection differentials and standardized selection gradients for all eight traits (see Fig. 2) for wild sunflowers grown near versus far from sunflower crops.

Variance of selection differentials did not differ near versus far ($F_{98,125}$ = 1.16, P = 0.422), but variance of selection gradients was greater near sunflower crops relative to far from sunflower crops ($F_{83,101}$ = 0.53, P = 0.003).

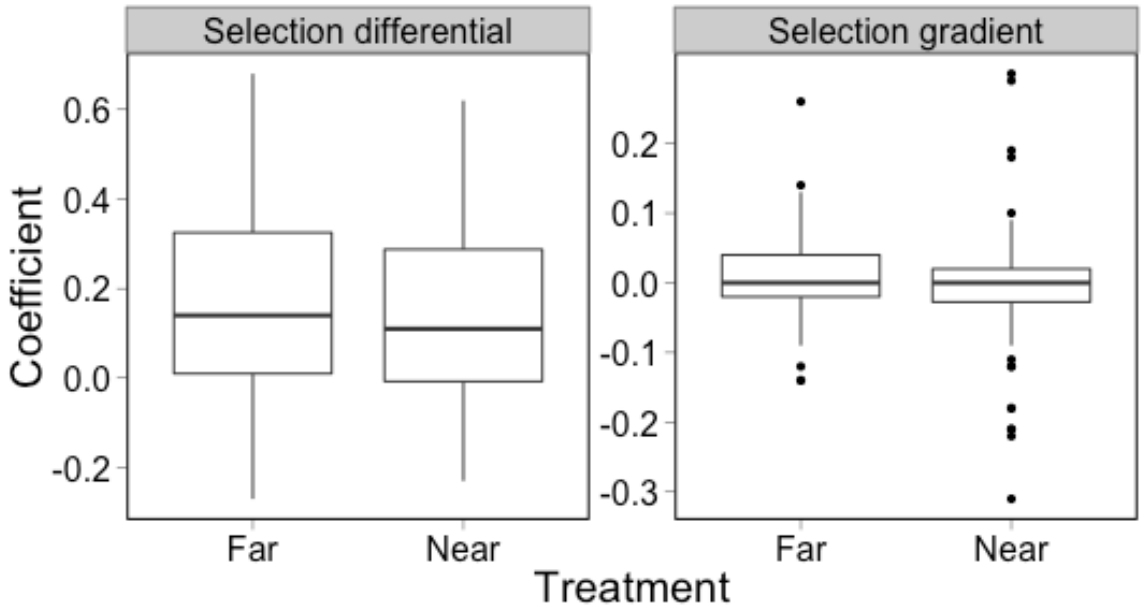
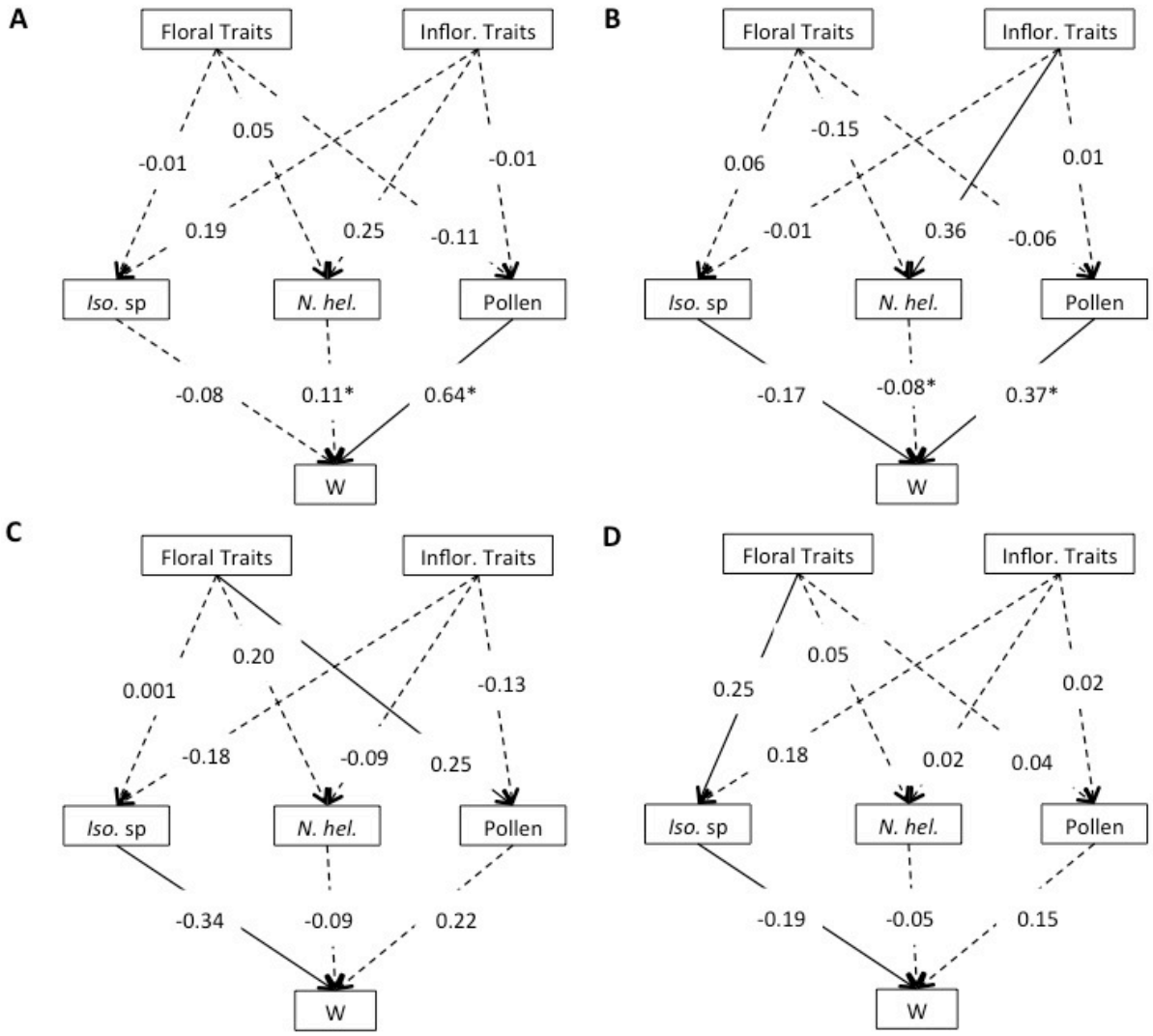


Figure 3.5. Path analysis results showing mutualist versus antagonist mediated selection on floral traits for Site 1 (A-B) and Site 2 (C-D) for Far (A,C) and Near (B,D) populations in 2011.

Correlations among exogenous variables, and plant number of inflorescences are not shown for clarity. * = Paths that differ ($P < 0.05$) Far versus Near within a site. Solid lines = significantly different from zero; dashed lines = not significantly different from zero. Antagonist seed predators, *Iso*. sp: *Isophrictis* sp.; *N. hel.* = *N. helianthi*. Mutualists, Pollen = pollen deposition. W = *H. a. texanus* relative fitness. Model fit: Site 1: $\chi^2 = 7.04, P = 0.134, df = 4, AIC = 111.0$. Site 2: $\chi^2 = 12.74, P = 0.239, df = 10, AIC = 104.7$.



3.11. Appendix A

Supplemental results: broad-sense heritability.

Methods

How variable are floral traits, and do they exhibit heritable broad-sense variation?

In 2009, we bulk collected seeds from ca. 80 plants in each of seven naturally occurring populations of *H. a. texanus*. Seeds from an additional two populations were collected in 2005 and 2006. The nine populations ranged from near Austin, TX to Houston, TX (Sites A-I; Fig. 1 in main text). In 2010, we planted a common garden at Katy Prairie Conservancy (29.964 N, 95.919 W) to estimate variation in floral traits and broad-sense heritability. We germinated a randomly chosen subset of seeds from each population and transplanted 20-148 seedlings from each of the nine populations of *H. a. texanus* (*N* = 775 total plants). All the traits measured for narrow-sense heritability (main text) were also measured for broad-sense heritability. We tested for significant overall variation in all traits among source populations using MANOVA, and each trait independently using ANOVAs. Broad-sense heritability (H^2) estimates the genotypic contribution to the phenotype (Falconer 1989), i.e., the genotypic variance over the phenotypic variance (V_G/V_P,), and was estimated here by using a linear random-effects model. From the model for each trait, we calculated variance components, and divided variation due to source population by total variation to get a measure of broad-sense heritability. Analyses were done in R v.2.14.1 (R Development Core Team 2011).

Results

Considered together, floral traits varied significantly among source populations (MANOVA; Pillai's trace = 0.83, $F = 4.79$, $P < 0.001$), and each of the nine traits independently varied significantly among source populations (ANOVAs: all $P < 0.001$; Fig. A1). Broad-sense heritabilities (H^2) ranged from 0.11 to 0.33 (Table A1).

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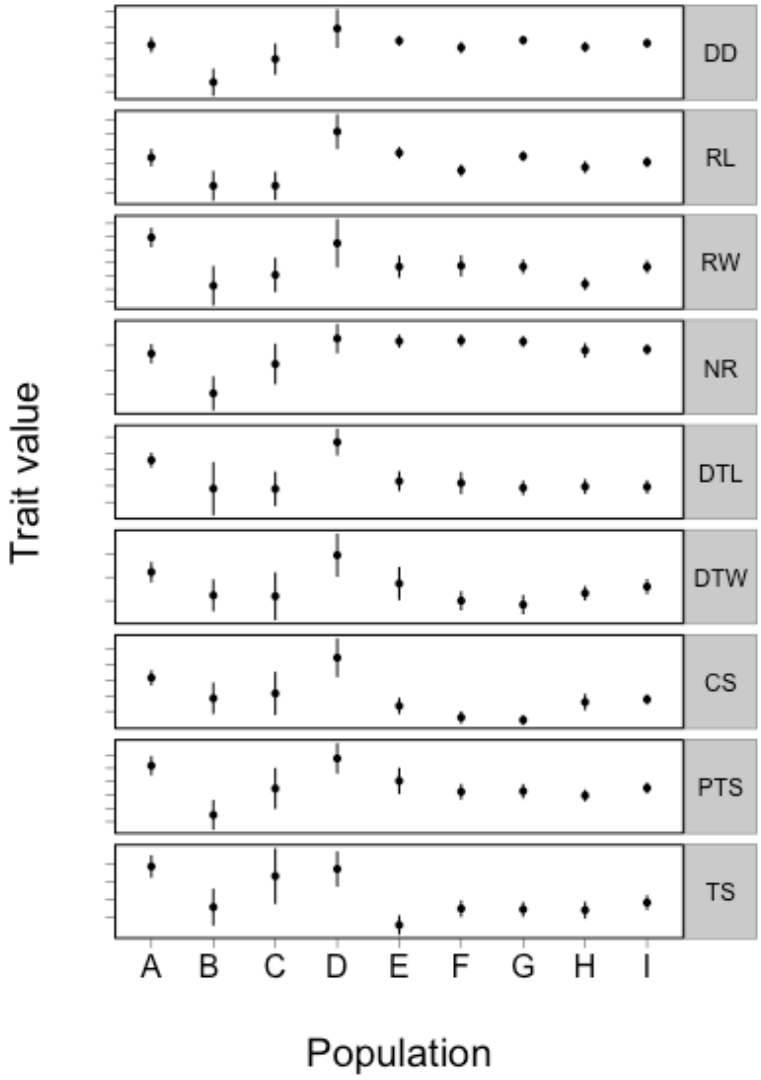
R: A language and environment for statistical computing, v.2.14.1 2.14.1, Vienna, Austria.

Table A1. Results of broad-sense heritability studies for four inflorescence, and five floral traits. Broad-sense values were estimated from nine natural populations grown in a common garden, (see Methods section for details). Tests are for heritability estimates significantly different from zero. See Fig. 2 in main text for diagram of floral traits.

	Broad-sense	
Trait	H^2	P
Inflorescence traits		
Disk diameter (DD)	0.22	< 0.001
Ray length (RL)	0.23	< 0.001
Ray width (RW)	0.14	< 0.001
No. rays (NR)	0.20	< 0.001
Floral traits		
Distal throat length (DTL)	0.15	< 0.001

Distal throat width (DTW)	0.11	< 0.001
Corolla lobe size (CS)	0.33	< 0.001
Proximal throat size (PTS)	0.19	< 0.001
Corolla tube size (TS)	0.25	< 0.001

Figure A1. Trait variation in four inflorescence traits and five disk floral traits in nine source populations of *H. a. texanus* in a common garden in 2010. Seeds for the common garden were collected in 2009 (see Fig. 1 in main text for geographic locations). Points are means, and bars are 95% confidence intervals around the mean. Populations were arranged roughly from northern to southern latitudes. Traits = DD: disk diameter; RL: max. ray length; RW: max. ray width; NR: no. or rays; DTL: distal throat length; DTW: distal throat width; CS: corolla lobe size; PTS: proximal throat size; TS: corolla tube size.



3.12. Appendix B

Table B1. Mean of pairwise correlations among floral traits. Values are Pearson coefficients. Correlation means greater than 0.5 are **bolded** to highlight the relatively stronger correlations. N = 25 plots for all correlation means.

Trait	Disc diam. (DD)	Max ray length	May ray width	No. rays	Distal throat length	Distal throat width	Corolla lobe size (CS)	Proximal throat size (PTS)

		(RL)	(RW)	(NR)	(DTL)	(DTW)		
Max ray length (RL)	**0.54**							
May ray width (RW)	0.44	**0.54**						
No. rays (NR)	**0.56**	0.20	0.05					
Distal throat length (DTL)	0.23	0.32	0.14	0.14				
Distal throat width (DTW)	0.15	0.14	0.19	-0.02	0.11			
Corolla lobe size (CS)	0.24	0.28	0.25	0.03	0.11	**0.65**		
Proximal throat size (PTS)	0.37	0.29	0.30	0.07	0.22	0.21	0.33	
Corolla tube size (TS)	0.33	0.27	0.25	0.12	0.32	0.10	0.25	**0.60**

Table B2. Results of analysis of covariance testing for differences in selection gradients due to proximity to sunflower crops and site in 2010. Data includes that for Sites 1, 2, 3, and 5 (see Fig. 1).

Variable	*ndf*	*ddf*	*F*	*P*-value
Site	3	6	0.89	0.498
Proximity	1	6	0.02	0.885
Site*Proximity	3	6	0.78	0.548
DD	1	832	11.42	**0.001**
DD*Site	3	832	0.44	0.722
DD*Proximity	1	832	0.17	0.681
DD*Site*Proximity	3	832	3.13	**0.025**
RL	1	832	0.22	0.640
RL*Site	3	832	1.62	0.184
RL*Proximity	1	832	5.29	**0.022**
RL*Site*Proximity	3	832	0.47	0.705
RW	1	832	0.54	0.462
RW*Site	3	832	0.21	0.892
RW*Proximity	1	832	2.75	0.098
RW*Site*Proximity	3	832	0.37	0.778
NR	1	832	0.94	0.332
NR*Site	3	832	1.01	0.388
NR*Proximity	1	832	0.05	0.826
NR*Site*Proximity	3	832	0.23	0.874
DTL	1	832	4.74	**0.030**
DTL*Site	3	832	0.10	0.959
DTL*Proximity	1	832	0.63	0.428
DTL*Site*Proximity	3	832	0.01	0.999
DTW	1	832	2.00	0.158
DTW*Site	3	832	2.17	0.090
DTW*Proximity	1	832	0.02	0.896
DTW*Site*Proximity	3	832	0.62	0.602
CS	1	832	0.48	0.487
CS*Site	3	832	3.10	**0.026**
CS*Proximity	1	832	3.26	0.071
CS*Site*Proximity	3	832	1.33	0.262
PTS	1	832	2.27	0.132
PTS*Site	3	832	0.81	0.490
PTS*Proximity	1	832	0.00	0.955
PTS*Site*Proximity	3	832	0.67	0.570
TS	1	832	1.14	0.285

Variable	*ndf*	*ddf*	*F*	*P*-value
TS*Site	3	832	0.05	0.985
TS*Proximity	1	832	1.23	0.267
TS*Site*Proximity	3	832	2.01	0.111
Plant vol.	1	832	321.65	**<0.001**
Plant vol.*Site	3	832	4.08	**0.007**
Plant vol.*Proximity	1	832	0.81	0.370
Plant vol.*Site*Proximity	3	832	5.16	**0.002**

Table B3. Results of analysis of covariance testing for differences in selection gradients due to proximity to sunflower crops, site, and year. Data includes that for Sites 1 and 2 for years 2010 and 2011 (see Fig. 1).

Variable	*ndf*	*ddf*	*F*	*P*-value
Year	1	7	0.58	0.471
Site	1	7	0.54	0.485
Proximity	1	7	0.00	0.991
Year*Proximity	1	7	0.48	0.512
Site*Proximity	1	7	0.18	0.687
Year*Site*Proximity	2	7	0.09	0.914
DD	1	1027	10.99	**0.001**
DD*Year	1	1027	0.50	0.481
DD*Site	1	1027	0.00	0.976
DD*Proximity	1	1027	2.39	0.122
DD*Site*Proximity	1	1027	0.88	0.349
DD*Year*Site*Proximity	3	1027	3.20	**0.023**
RL	1	1027	0.81	0.369
RL*Year	1	1027	0.12	0.725
RL*Site	1	1027	0.12	0.724
RL*Proximity	1	1027	8.88	**0.003**
RL*Site*Proximity	1	1027	0.73	0.394
RL*Year*Site*Proximity	3	1027	0.53	0.659
RW	1	1027	0.18	0.669
RW*Year	1	1027	0.05	0.821
RW*Site	1	1027	4.86	**0.028**
RW*Proximity	1	1027	2.73	0.099
RW*Site*Proximity	1	1027	0.00	0.955
RW*Year*Site*Proximity	3	1027	1.28	0.280
NR	1	1027	14.19	**<0.001**
NR*Year	1	1027	0.92	0.338
NR*Site	1	1027	1.91	0.167
NR*Proximity	1	1027	1.42	0.235
NR*Site*Proximity	1	1027	0.00	0.967
NR*Year*Site*Proximity	3	1027	0.49	0.692
DTL	1	1027	2.79	0.095
DTL*Year	1	1027	0.61	0.437
DTL*Site	1	1027	0.03	0.868
DTL*Proximity	1	1027	0.17	0.679
DTL*Site*Proximity	1	1027	1.00	0.318
DTL*Year*Site*Proximity	3	1027	0.43	0.732
DTW	1	1027	1.74	0.188
DTW*Year	1	1027	2.81	0.094

Variable	*ndf*	*ddf*	*F*	*P*-value
DTW*Site	1	1027	2.78	0.096
DTW*Proximity	1	1027	0.69	0.408
DTW*Site*Proximity	1	1027	0.00	0.992
DTW*Year*Site*Proximity	3	1027	1.78	0.150
CS	1	1027	1.15	0.284
CS*Year	1	1027	0.59	0.444
CS*Site	1	1027	2.58	0.108
CS*Proximity	1	1027	0.25	0.619
CS*Site*Proximity	1	1027	0.66	0.417
CS*Year*Site*Proximity	3	1027	2.27	0.079
PTS	1	1027	0.55	0.457
PTS*Year	1	1027	0.01	0.913
PTS*Site	1	1027	1.12	0.291
PTS*Proximity	1	1027	3.56	*0.059*
PTS*Site*Proximity	1	1027	0.65	0.422
PTS*Year*Site*Proximity	3	1027	0.82	0.486
TS	1	1027	1.15	0.283
TS*Year	1	1027	0.16	0.687
TS*Site	1	1027	0.07	0.786
TS*Proximity	1	1027	0.01	0.918
TS*Site*Proximity	1	1027	1.57	0.211
TS*Year*Site*Proximity	3	1027	1.26	0.287
Plant vol.	1	1027	669.46	**<0.001**
Plant vol.*Year	1	1027	46.66	**<0.001**
Plant vol.*Site	1	1027	14.75	**<0.001**
Plant vol.*Proximity	1	1027	1.80	**0.180**
Plant vol.*Site*Proximity	1	1027	33.58	**<0.001**
Plant vol.*Year*Site*Proximity	3	1027	6.33	**<0.001**

Table B4. Selection differentials for floral traits. Sample sizes (no. plants) for the calculations of selection differentials range 13-139 (mean: 60). See Fig. 1 in main text for sites and seed sources. Abbreviations: DD: disk diameter; RL: max. ray length; RW: max. ray width; NR: no. or rays; DTL: distal throat length; DTW: distal throat width; CS: corolla lobe size; PTS: proximal throat size; TS: corolla tube size.

Site	Seed Source	Proximity	DD	RL	RW	NR	DTL	DTW	CS	PTS	TS
2010											
Site 1	C	Far	0.00	0.10	0.10	0.04	-0.05	-0.14	-0.10	-0.19	-0.06
		Near	0.18	0.18	0.19	0.17	-0.18	-0.06	-0.10	0.14	-0.01
	B	Near	0.46***	0.24**	0.12	0.42***	0.09	-0.08	0.05	0.21*	0.17
Site 2	C	Far	0.50***	0.31**	0.09	0.34**	0.19	0.13	0.02	0.08	0.05
		Near	0.44***	0.38**	0.37**	0.35**	0.27*	0.01	-0.07	0.05	0.07
	B	Far	0.68***	0.54***	0.37**	0.65***	0.22†	0.14	0.35**	0.33**	0.36**
		Near	0.61***	0.40***	0.43***	0.36**	0.16	0.02	0.11	0.29**	0.36**
Site 3	C	Far	0.43***	0.46**	0.30*	0.32*	0.01	-0.14	-0.08	0.01	-0.19
		Near	0.11	0.06	0.03	0.10	-0.02	-0.09	-0.16	-0.18	-0.20
	B	Far	0.37*	0.47**	0.07	0.36*	0.25	-0.01	0.27	0.10	0.07
		Near	0.62***	0.32**	0.31**	0.44***	0.39**	-0.10	0.05	-0.06	0.12
Site 4	C	Near	0.33*	0.26	0.28†	0.22	-0.05	-0.02	-0.18	0.09	0.07
	B	Near	0.33	0.58*	0.45	-0.11	0.59*	0.16	0.21	0.23	0.36
Site 5	C	Far	0.27†	0.30*	0.26	0.19	0.05	0.24	0.21	-0.17	0.06
		Near	-0.02	0.01	-0.14	-0.08	0.33†	0.10	-0.02	-0.05	-0.11
	B	Far	-0.01	-0.20	-0.27*	-0.09	-0.09	0.01	0.01	0.00	0.03
		Near	0.34**	0.20	0.06	0.29*	-0.08	0.10	0.06	0.12	0.17
2011											
Site 1	C	Far	0.40***	0.42***	0.36**	0.29**	-0.18	-0.15	0.25*	-0.12	-0.10

Site	Seed Source	Proximity	DD	RL	RW	NR	DTL	DTW	CS	PTS	TS
		Near	0.00	-0.01	-0.03	-0.18	-0.23	-0.12	-0.08	0.03	0.05
Site 2	C	Far	0.48***	0.29*	0.33**	0.28*	0.10	0.21	0.05	0.13	0.36**
		Near	0.35†	0.39*	0.09	0.29	0.21	0.14	0.30	0.11	0.22
	B	Far	0.01	0.04	-0.09	0.16	0.20	-0.02	0.09	0.20	0.14
		Near	0.44***	0.26*	0.22*	0.43***	0.10	0.00	0.04	0.14	0.01

† P < 0.06; * P < 0.05; ** P < 0.01; *** P < 0.001

Table B5. Selection gradients from phenotypic selection analysis testing for selection on floral traits in each of 25 plots. See Table B4 for more information.

Site	Seed Source	Proximity	DD	RL	RW	NR	DTL	DTW	CS	PTS	TS
2010											
Site 1	C	Far	0.00	0.02	-0.01	0.00	-0.01	-0.02	0.01	-0.02	-0.01
		Near	0.10	-0.12	0.03	-0.08	-0.02	-0.21	0.18	-0.03	-0.07
	B	Near	0.04†	-0.02	0.01	0.03†	-0.01	-0.04*	0.02	-0.01	0.01
Site 2	C	Far	0.05**	0.03	-0.02	0.00	-0.04†	0.01	-0.03†	0.01	-0.01
		Near	-0.01	0.01	0.00	0.01	0.00	-0.01	-0.01	-0.03	0.03
	B	Far	0.11	0.07	-0.05	0.10	0.02	-0.01	0.05	0.06	-0.09
		Near	0.01	0.00	0.02	0.01	-0.01	0.00	-0.02	0.01	0.00
Site 3	C	Far	-0.04	0.13	0.05	0.06	--	--	--	--	--
		Near	0.29*	0.00	-0.02	-0.09	-0.07	0.05	-0.09	-0.18	-0.18*
	B	Far	-0.14	0.26*	-0.07	0.08	--	--	--	--	--
		Near	0.04	0.00	0.04*	0.02	0.00	-0.01	-0.01	0.01	-0.03†
Site 4	C	Near	0.30	-0.04	-0.06	-0.11	0.03	0.09	-0.21	-0.07	0.05
	B	Near	--	--	--	--	--	--	--	--	--
Site 5	C	Far	0.12	0.02	0.05	-0.06	-0.01	0.04	0.11	-0.14	0.04
		Near	-0.31	0.06	-0.22	0.19	--	--	--	--	--
	B	Far	0.07	-0.04	-0.12	-0.06	-0.03	-0.03	0.08	0.02	-0.01
		Near	0.02	-0.03	0.01	-0.01	0.00	0.00	0.00	-0.01	-0.01
2011											
Site 1	C	Far	0.04	0.04	-0.03	0.00	-0.01	-0.03*	0.02	-0.04*	0.00
		Near	-0.01	-0.01	0.01	0.03**	0.01	0.01	-0.01	0.02	-0.02
	B	Far	0.14**	0.03	-0.06	0.04	-0.02	0.04	-0.04	-0.05	0.00
		Near	0.03	-0.02	-0.04	-0.06	--	--	--	--	--
Site 2	C	Far	0.04	0.00	0.01	0.01	-0.01	0.01	0.00	-0.02	0.01
		Near	-0.03	0.03	-0.02	0.06**	--	--	--	--	--
	B	Far	-0.02	0.01	0.02	0.02	--	--	--	--	--
		Near	0.00	-0.05	0.05	0.09*	0.01	0.03	-0.12**	0.04	0.01

† P < 0.06; * P < 0.05; ** P < 0.01; *** P < 0.00

4. How context-dependent are species interactions?

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3.13. Abstract

Species interactions vary in net effects in sign (-, 0, +) and magnitude. This variation is commonly described as context-dependency: the sign and/or magnitude of an interaction change as a function of the biotic or abiotic context in which the interaction is embedded. Context-dependency in interaction outcomes influences the dynamics of populations, the structure of food webs, and the evolution of species traits. While such variation appears to be common, ecologists typically focus on understanding factors that influence mean interaction outcomes rather than variance. Here, we used meta-analysis to quantify variation in interaction outcomes across 353 published papers. We tested whether variation in sign or magnitude of interaction outcomes differed among predation, competition, and mutualism and across contexts, including abiotic gradients, multiple sites (spatial), multiple dates (temporal), variation in species identity, and variation in the presence of a third party species. While the magnitude of variation in outcomes did not significantly

differ among species interactions, a change in interaction sign (-, 0, +) was most likely for mutualism, least likely for predation, and intermediate for competition. Differences among species interactions also depended on the context. Competition was more variable in magnitude than mutualism through time. Along abiotic gradients and through time, competition and mutualism were more likely to change sign than predation. In contrast, predation was more variable than competition and mutualism when species identity generated the context-dependency. Overall, both the magnitude and sign of species interactions varied the most as a function of the identity of the interacting species and least as a function of the presence/absence of a third party. We conclude that, on average, mutualism, competition, and predation are characterized by similar magnitudes of variation in effect size, but mutualism has the highest propensity to change sign and predation the lowest.

3.14. Introduction

Variation in the outcome of species interactions is common in nature. This variation is commonly described as context-dependency: the sign and/or magnitude of an interaction change as a function of the biotic or abiotic context in which the interaction is embedded. A notable example of variation in interaction outcomes is found in plants. At 11 sites around the globe, interspecific interaction outcomes between plant species are context-dependent on elevation: competition dominated at low elevations where abiotic stress was relatively low, but facilitative interactions

dominated at high elevations where abiotic stress was relatively high (Callaway et al. 2002). In addition to competition/facilitation interactions, variation in predator-prey interaction strengths is common. Menge et al. (1994) demonstrated that variation in interaction strength between Pisaster seastars and Mytilus mussels occurred across three spatial scales, from < 10 m to 10's of km; variation in interactions were greater in space than time, and abundance was important as a driver of variable strengths. These and many other studies (e.g., Cushman and Whitham 1989, Thompson and Cunningham 2002, Pennings and Silliman 2005, Navarrete and Berlow 2006) have documented variation in species interactions.

Variation in interaction outcomes can influence both the ecological and evolutionary dynamics of populations and communities. On ecological time scales, variation in interaction outcomes result in variation in population growth. For example, insect herbivory on cholla cactus in the Chihuahuan Desert varied among populations along an elevational gradient: stronger herbivory at low elevation weakened cactus population growth, while weaker herbivory at high elevation lead to stronger cactus population growth (Miller et al. 2009). In addition, variation in interaction outcomes can affect community properties, such as food web structure. Species interactions that are less variable may lead to greater food web compartmentalization; in contrast, when species interactions are more variable, species interactions that form links in food web compartments may stray outside of compartments, decreasing compartmentalization of the web (Miller and Travis 1996, Travis 1996), and decreasing food web stability (Kokkoris et al. 2002, Stouffer and Bascompte 2011). On evolutionary time scales, variation in the outcomes of

species interactions influences natural selection on traits that mediate interactions (Thompson 2005). For example, variation in the strength of protection conferred by ants resulted in different selection pressures on extrafloral nectary traits that attract ants to wild cotton plants in the Sonoran Desert (Rudgers and Strauss 2004). These examples illustrate that variation in interaction outcomes is important for population and community ecology, and evolutionary processes.

Despite the potential importance of context-dependency in the magnitude and sign of species interactions, ecologists have traditionally focused on the mean direction and magnitude of the impact of one species on another (Benedetti-Cecchi 2003, Fraterrigo and Rusak 2008). As a consequence, the magnitude of variation in species interactions and drivers of this variation have not been as well characterized as the mean (Benedetti-Cecchi 2000, Inouye 2005, Agrawal et al. 2007). Here, we explore variation in species interaction outcomes across types of species interactions and examine the drivers of this variation.

A key question is whether certain types of species interactions vary in outcome more than others. The mutualism-parasitism continuum hypothesis (Johnson et al. 1997, Karst et al. 2008) proposes that mutualisms commonly grade into parasitism as contexts vary and suggests that positive species interactions are more likely to show context-dependency than antagonisms. While some biological phenomena display increasing variance with larger means (Taylor 1961), empirical evidence suggests that species interactions with weaker average effect sizes are weaker precisely because they are highly variable in magnitude across contexts

(Berlow 1999). Previous reviews have found that mean interaction outcomes are largest in predation, less in competition, and the least in mutualism (Sih et al. 1985, Gurevitch et al. 2000, Morris et al. 2007), suggesting that mutualisms will have more variable interaction outcomes than antagonisms, if in fact weaker mean interactions are more variable (Berlow 1999). An alternative hypothesis that leads to the same prediction involves the flow of energy: predation involves a more direct flow of energy than does competition or mutualism. Competition and mutualism are often mediated by an external resource or condition (e.g., exploitative competition for nutrients or light; Kersch and Fonseca 2005), bi-directional flows of energy, or the presence of an additional species (e.g., herbivores required for benefits to accrue to plants in ant-plant protection mutualism), opening up the interaction to greater opportunities for variation. Thus, we predict that mutualisms will be the most variable interaction types.

Variation in interaction outcomes often occurs along gradients, such as abiotic conditions (e.g., temperature; Daskin and Alford 2012), the abundance of a third party species (e.g., shared predator), and shifting identities of the individual participants. For example, Del-Claro and Oliveria (2000) showed that the outcome of a protection mutualism between treehoppers and ants varied over days and years. In addition, a third party (spiders) external to the pairwise protection interaction varied in abundance through time, further varying the outcomes of treehopper-ant interactions. Often variation is studied via gradients in space and time, which may represent a number of changes in context. Here, we refer to all these as context gradients, the gradients along which species interactions occur.

Although much research has documented variation in species interactions (e.g., Menge et al. 1994), the relative importance of different kinds of gradients for generating variation in species interactions is largely unknown.

In addition to the gradients along which interactions occur, interactions take place in different ecosystems, as well as different experimental locations. Previous studies have documented that species interactions do (predation; Preisser et al. 2005) or do not (herbivory; Hillebrand 2009) vary in mean outcomes among ecosystems. It is not known if variation in outcomes differs among ecosystems. The ecosystems we consider here are terrestrial, freshwater (lentic and lotic), and marine. In field settings, we expected that studies in terrestrial systems would be more variable than both freshwater and marine systems because abiotic conditions are expected to vary more in terrestrial systems (Sculthorpe 1967). In addition to ecosystem variation, we expected greater variation in field studies than in greenhouses, and the least variation in laboratory studies, reflecting a decreasing gradient of control over variables that could generate context-dependency, and matching similar patterns for differences in mean species interaction outcomes among study locations (Hillebrand 2009).

Using meta-analysis of data from 353 published papers, we compared the magnitude and sign of variation in species interaction outcomes among three major classes of species interactions: competition, predation, and mutualism. We quantified context-dependency using two response variables: the coefficient of variation (CV) of interaction outcomes among contexts (e.g., years or sites) within a

study; and the change in sign of the interaction outcome (+, 0, -) across contexts within a study. CV quantifies the amount of total variation with respect to the mean outcome, while sign-change reports only whether interaction outcomes changed between any of positive (+), neutral (0), or negative (-) interactions, without regard for the magnitude of variation. We used meta-analysis to address three questions: (1) Do types of species interactions (competition, predation, mutualism) and context gradients differ in the magnitude of variation in interactions? (2) Do types of species interactions (competition, predation, mutualism) and context gradients differ in their propensity for sign change of the interaction? (3) Does variation in magnitude of variation or propensity for sign change vary consistently with study location or ecosystem type? and (4) Is magnitude of variation or propensity for sign change biased by experimental methods?

3.15. Materials and Methods

4.3.1. Literature search and dataset description

We searched for relevant papers using two general methods. First, we searched for papers in previous meta-analyses examining competition, predation, or mutualism (Gurevitch et al. 1992, Chase et al. 2002, Kaplan and Denno 2007, Holt et al. 2008, Chamberlain and Holland 2009). Second, we searched Web of Science for papers on predation (keywords: "(pred* and prey) AND experiment*"), competition ("competition AND experiment*"), and mutualism ["(mutualis* OR pollinat* OR mycorrhiza* OR rhizobi* OR endophyte) AND experiment*"], refined to include

"ecology" papers only. For both methods of searching, we used the following selection criteria: (1) The study measured the responses of individual organisms to experimental manipulation of one of the following interaction types: interspecific competition, predation, or mutualism. Observational studies that performed natural, or uncontrolled experiments were not included; this does exclude many studies, but including only manipulative experimental studies allows us to infer causality (e.g., species identity was manipulated in a study, allowing us to conclude that interaction outcome variation is associated with species identity). (2) The study measured response variables under more than one context (e.g., in multiple years or multiple competitor species); this restricted our set to studies informative on context-dependency. (3) The study provided estimates of the mean response, sampling dispersion around the mean (e.g., standard deviation or standard error), and sample sizes for each treatment.

When multiple response variables (e.g., growth rate and fecundity) were reported for the same species interaction, we recorded values for all response variables. We took the mean across response variables to get the average of different components of a species response to a species interaction. Although independent controls for each experimental treatment are ideal, we collected data for experiments that did not have separate controls for each treatment level, and used this as a factor in the analysis (see Table 1). When data were available for multiple dates we collected all data and averaged the outcomes, unless we were examining temporal variation in outcomes. When data were replicated over multiple factors, we collected all data and averaged across the other factors other

than contexts of interest. For predation studies, we excluded all studies (n = 3) from the final dataset that measured the response in the predator - so all predation studies measured the response in the prey.

The final dataset included 353 papers. Some analyses in this paper used subsets of the complete dataset. Appendix A provides references for all papers used in the analyses.

4.3.2. Independent variables

Data on several independent variables were collected: species interaction type, context gradient (see next paragraph), study location, and ecosystem (Table 1). Species interaction type had one of three values: competition, predation, or mutualism. Study location was categorized as one of four values: laboratory, greenhouse, aquatic or terrestrial mesocosm, or field study. Ecosystem held one of three values: marine, freshwater (includes lotic and lentic), or terrestrial. Data were categorized into one of five gradients of context-dependency: abiotic, spatial, temporal, third party presence, or species identity (Table 1). *Abiotic variation* in outcomes occurred when outcomes were measured across either an environmental gradient or in different habitat types (e.g., sand and mud habitat; Bonsdorff et al. 1995). Although different habitats varied not only in abiotic variables but in biotic ones as well, with no information with which to gauge the change in biota, we grouped these studies into abiotic contexts. *Spatial variation* in outcomes included studies for which outcomes were measured across different sites, explicitly examining different physical locations instead of different habitat types within a

single site (Hindell et al. 2002). Temporal variation in outcomes occurred when outcomes were measured at multiple points in time, ranging from hours to years (e.g., Barnes and Archer 1999). *Third-party presence variation* captured studies of the same pairwise interaction in the presence and absence of an additional species (e.g., competition between plant species in the presence and absence of aphids; Schadler et al. 2007). *Species identity variation* occurred when outcomes were assessed for a focal species interacting with a suite of different species (e.g., interactions between one focal species, while identity of second species varies; Smith 2005), and included studies in which multiple predator species interacted with a single prey species. Both third party presence and species identity capture strong biological contingency in outcomes. There were too few studies of intraspecific variation (body size, developmental stage) and abundance (of one species) across all interaction types to permit analysis of these context types.

In addition, we collected data on potential bias variables: paired controls, average duration of the study, sample size per treatment combination, the year the study was published, and journal impact factor during the year the study was published (Table 1). The paired controls variable was scored as a binary variable, taking a value of 0 if the study did not use separate controls for each experimental treatment, and a value of 1 if the study did use separate controls. We expected that studies that did not use controls for each experimental treatment may bias results, but we had no expectation of direction. Average duration of the study was recorded as number of days from the start of the experiment to the day of the response variable was measured. We expected that studies conducted over a longer period

would be more likely to include extreme interaction outcomes, leading to greater variation. Sample size was calculated as the mean sample size across the control and experimental treatments. Likewise, we expected that studies with greater sample sizes would be more likely to capture strong variation in interaction outcome. We recorded the year each paper was published. We expected no biological differences in effect sizes through time, so variation in effect sizes through time would indicate publication bias. Journal impact factor is the ISI journal impact factor during the year the study was published, and was derived from Journal Citation Reports ® (prepared by Thomson Reuters ®, Inc., Philidelphia, PA, USA). We expected lower variability for studies published in higher impact journals, which may select for studies with larger, less variable effects.

There were additional variables we investigated, which did not effect our results, and are discussed in Appendix B.

4.3.3. Effect size calculations

We calculated relative interaction intensity as $RII = \bar{x}_C - \bar{x}_E / \bar{x}_C + \bar{x}_E$, and variance of *RII* following Armas *et al*. (2004). *RII* gives the difference in magnitude of the response variable in control and experimental treatments, standardized by the sum magnitude. We chose *RII* because of its excellent statistical properties. It is bounded between 1 and -1, is symmetrical around zero, and is negative for negative effects on the focal species and positive for positive effects on the focal species (the focal species is the one on which the response variable was measured). The symmetry was particularly important as it allowed us to compare the effect sizes of

positive and negative interactions on the same scale by taking the absolute value of RII. Hedges' d was not useful for this study because of its unbounded range.

We examined variation of *RII* in two ways. First, we calculated the coefficient of variation (CV*) for *RII* among records within a study as: $CV_{RII} = (\sigma_{RII}/\bar{x}_{RII})\times 100$, where σ_{RII} is the standard deviation *RII* across all contexts within a study, and $\bar{x}_{RII}$ is the mean of *RII* for all contexts within a study or species. We then calculated the unbiased estimator of CV_{RII} as $CV^*_{RII} = (1 + 1/4n)\, CV_{RII}$, which corrects for small sample sizes (Haldane 1955), an important correction because studies varied widely in the number of contexts examined (mean ± 1 s.e.m. = 3.86 ± 0.18, range = 2-31). CV^*_{RII} has a variance of $v_{CV^*} = \left((1 + 1/4n)s_{CV}\right)^2$ (Sokal and Rohlf 1969), where n is the number of independent replicates (i.e., "contexts") used to calculate the CV^*_{RII} for each study. There were no significant differences in the number of contexts examined among the types of species interactions ($F_{2,338} = 0.43$, $P = 0.65$). However, some context gradients had greater representation than others ($F_{4,338} = 12.73$, $P < 0.0001$). Specifically, studies on third parties had the fewest contexts examined, followed by abiotic gradients, then spatial studies. Studies of species identity and temporal variation had the most contexts examined, significantly more than abiotic or third party. For meta-analysis on CV^*_{RII}, we weighted studies by the product of the sample size per treatment combination and the number of contexts examined, which gave the largest weights to studies with high replication both within treatments and across contexts (Hedges and Olkin 1985). We attempted meta-analysis using the inverse of the variance of CV^*_{RII}, but this resulted in an inverse correlation between the weight and the CV^*_{RII}, meaning that studies with the largest

CV^*_{RII} always had the smallest weights. Second, we determined whether effect sizes within a study had a change in sign (hereafter 'sign change') of the interaction, either between zero (0) and negative (-), zero (0) and positive (+), or negative (-) and positive (+). Sign change differs from CV^*_{RII} in that it more explicitly quantifies whether the interaction changed in direction, not just magnitude (e.g., see Bronstein 1994). We averaged effect sizes within response variables, calculated the sign of the interaction outcome (-1, 0 1), then determined whether the sign changed for any of the response variables (no change in any response variable = 0, change = 1). To calculate sign change we first determined for each effect size (*RII*) if it significantly differed from zero using a 95% confidence limit, as $RII \pm 1.96\left(\sigma_{RII}/\sqrt{n}\right)$, where σ_{RII} is the standard deviation of *RII* and *n* is the sum of replicates from each record. Effect sizes that were significantly different from zero in the negative direction were assigned a -1, those in the positive direction were assigned a 1, and those not different from zero were assigned zero. Ultimately, all records were assigned a vector taking one of three values: -1, 0, or 1. Sign change is a binary response variable, and took a value of 1 if this vector included more than one of the three values in the vector (-1, 0, 1), and 0 if not.

4.3.4. Statistical analyses

Because some studies explored multiple gradients of context-dependency (e.g., variation in both time and space), we maximized representation across context types by using an algorithm to pick data for the least represented context for any study that examined multiple types – resulting in one record per study. Thus,

analyses are not confounded by nonindependence due to study. Five studies were excluded from the analyses due to extremely high weights resulting from very low variance or high sample sizes, resulting in a final sample of 353 studies. We did not account for information on the phylogenetic history of the taxa because this would have required arbitrary choices about which taxon (e.g., predator vs. prey) to track.

1) *Do types of species interactions (competition, predation, mutualism) and context gradients differ in the magnitude of variation*? Data were analyzed with weighted general linear models that included the fixed effects of context type (Table 1) and species interaction type (predation, competition, mutualism), as well as their interaction (SAS v. 9.2, SAS Institute, Cary, NC). CV^*_{RII} was log-transformed to achieve normality of residuals and homogeneity of variances. When the interaction type × context effect was significant, we decomposed the differences among species interaction types with *post-hoc* Tukey HSD tests within each context.

2) *Do types of species interactions (competition, predation, mutualism) and context gradients differ in their propensity for sign change of the interaction*?

For sign change (binary response variable) we specified a binomial distribution and logit link function. We followed the same analysis workflow as above for Q1.

3) *Does variation in magnitude of variation or propensity for sign change vary consistently with study location or ecosystem type*? We included each factor (study location and ecosystem) individually in general linear models to test for statistical interactions with the species interaction type and the context type. These analyses

tested whether differences in context-dependency among the three types of species interactions varied with study location or ecosystem (Table 1). Examining each factor individually allowed us to maximize replication because many factors were not recorded for every study. If factors interacted with species interaction type or context type, we used sequential Bonferroni adjustments to *P*-values when decomposing interactions.

4) Is magnitude of variation or propensity for sign change biased by experimental methods? These analyses tested whether differences in context-dependency among the three types of species interactions varied with whether or not the study used paired controls, the average duration of the study (days), the sample size per treatment combination, the year in which the study was conducted, and the journal impact factor during the year the study was published (Table 1). We followed statistical approaches as in Q3 above.

3.16. Results

4.4.1. *Do types of species interactions (competition, predation, mutualism) and context gradients differ in the magnitude of variation*?

CV^*_{RII} spanned six orders of magnitude (range: 0.8 – 18,227). On average, there were no significant differences among species interaction types in interaction outcome magnitude, CV^*_{RII} (Fig. 1A, Table 2). This result is in conflict with our prediction that mutualisms would be more variable than antagonistic interactions. In fact, the type of context was a more significant driver of CV^*_{RII} than was the type

of species interaction (Fig. 3, Table 2). CV^*_{RII} was greatest across the species identity axis, and was 123% greater than temporal gradients and 257% greater than third party presence (Fig. 3). Species interactions did differ in their degree of context dependency along one context axis (Fig. 2, interaction type × context type, $P = 0.004$, Table 2). Along the temporal axis CV^*_{RII} was 160% greater for competition than mutualism, whereas competition did not differ from predation, nor did predation differ from mutualism (Fig. 2). Two representative studies highlight this result. In a competition study, growth of *Carex vaginata* (Cyperaceae) was quite variable in response to competition with *Dryas octopetala* (Rosaceae) over time ($CV^*_{RII} = 139$) (see Fig. 2 in Klanderud 2005). However, growth of *Sporobolus wrightii* plants (Poaceae) inoculated with mutualistic mycorrhizal fungi resulted in much less temporal variation ($CV^*_{RII} = 33$) in the interaction outcome than the competition average (see Table 2 in Richter and Stutz 2002). Predation and mutualism showed their greatest context-dependency across species identity gradients, whereas competition was about equally variable across species identity and temporal gradients (Fig. 2).

4.4.2. Do types of species interactions differ in propensity for changing the interaction sign?

On average, the likelihood that the interaction changed in sign across contexts was highest for mutualism, intermediate for competition, and lowest for predation (Fig. 1B , Table 2). Unlike the magnitude of variation, this result is consistent with our prediction that mutualism would be the most variable species

interaction type. Three studies from out dataset illustrate these results. In a mutualism study, the change in interaction outcome sign likelihood was about 67% when an arbuscular mycorrhizal species interacted with a plant species (*Leucanthemum vulgare*; Asteraceae) over four years (Gange et al. 2003). Although no studies fell near the competition mean for sign change (61%; Fig. 1), competitive interactions between grasses and five different species of shrubs and trees changed had a 67% likelihood of sign change (D'Antonio et al. 1998). In a predation study, interactions between *Basiaeshna janata* (Odonata: Aeshnidae) dragonfly predators and four species of damselfly prey resulted in the least variation among the three species interaction types (50%; McPeek 1998).

Variation in the sign of the interaction did not correspond closely to variation in the magnitude of the effect size, as captured by CV^*_{RII} (compare Figs. 1A–B). Like CV^*_{RII}, sign change varied significantly among context types. In particular, like CV^*_{RII}, the greatest propensity for sign change in interactions occurred with species identity. Mean sign change was the smallest for studies of third party presence, while temporal, spatial, and abiotic contexts were intermediate. Like CV^*_{RII}, the sign change responded to the combined influence of species interaction × context type (Fig. 2, $P < 0.0001$, Table 2). Under variable abiotic conditions, a change in the sign of the interaction was more likely for competition and mutualism than for predation (Fig. 2). In contrast, variation in space and in the presence of a third party species had stronger effects on the sign of mutualism than on the sign of negative interactions (predation and competition). Predation was more likely to change sign

than competition or mutualism with variation in species identity, and was less likely than competition and mutualism to change sign over time (Fig. 2).

4.4.3. Does variation in magnitude of variation or propensity for sign change vary consistently with study location or ecosystem type?

We expected that experiments conducted in more controlled locations (e.g., laboratories) would show less variable species interaction outcomes than field locations. Although location of the study had no significant influence on the CV^*_{RII} ($F_{3,347} = 1.50$, $P = 0.214$), location of the study did influence sign change ($\chi^2 = 454.14$, $P < 0.0001$). In opposition to our expectation, sign change differed among all locations, with the greatest sign change in laboratories, less in greenhouses, even less in field studies, and the least in outdoor tanks (terrestrial or aquatic).

CV^*_{RII} showed no significant divergence among ecosystems (ecosystem type, $F_{2,343} = 0.43$, $P = 0.649$; interaction type × ecosystem type, $F_{3,343} = 0.84$, $P = 0.471$). However, sign change did differ among ecosystems ($\chi^2 = 118.98$, $P < 0.001$). Sign change was 31% more frequent in terrestrial than in marine systems (Tukey test; $P < 0.0001$), as we had predicted. Terrestrial ecosystems did not differ from freshwater systems ($P > 0.05$), but freshwater systems were 26% more likely to change sign than marine systems ($P < 0.0001$).

4.4.4. Is magnitude of variation or propensity for sign change biased by experimental methods?

Studies that did not use a separate control treatment for each context in the experiment were 19% more likely to detect a sign change than those with separate controls (χ^2 = 35.62, P < 0.001), but this element of the design did not significantly affect CV*$_{RII}$ ($F_{1,349}$ = 0.24, P = 0.627). Surprisingly, average duration of the study had no effect on CV*$_{RII}$ (β = 0.02, P = 0.750), but was positively related to sign change (β = 0.13, P < 0.0001), as we expected.

The year in which the study was published had no influence on CV*$_{RII}$ ($F_{1,351}$ = 0.65, P = 0.421), but did influence sign change. Specifically, there was no main effect of year of the study on sign change (χ^2 = 0.00, P = 0.959), but there were significant differences in the relationship between year and sign change across different interaction types (χ^2 = 25.07, P < 0.001), context types (χ^2 = 63.39, P < 0.001), and combinations of interaction type × context type (χ^2 = 162.60, P < 0.001). Mutualism studies published more recently were more likely report a change in sign temporally (β = 0.04, P = 0.0001), while competition (β = -0.19, P < 0.0001) and predation (β = -0.13, P < 0.0001) studies published more recently were less likely to find a sign change. In addition, mutualism studies published more recently were more likely to document a change in sign with species identity (β = 0.06, P < 0.0001), while competition (β = -0.15, P < 0.0001) and predation (β = -0.08, P < 0.0001) studies published more recently were less likely to show a change in sign. Similarly, mutualism studies published more recently were more likely to document a change in sign with third party presence (β = 0.03, P = 0.041), while competition studies were less likely to show a change in sign (β = -0.10, P = 0.0004).

We expected that studies published in journals with higher impact factors would have less variable effect sizes. Journal impact factor had no significant effect on CV^*_{RII} ($F_{1,348}$ = 0.74, P = 0.391), but did influence sign change. There was no main effect of impact factor on sign change (χ^2 = 0.61, P = 0.433), but there were significant differences in the relationship between impact factor and sign change across different interaction types (χ^2 = 145.44, P < 0.001), context types (χ^2 = 155.11, P < 0.001), and combinations of interaction type × context type (χ^2 = 349.23, P < 0.001). Mutualism studies along the temporal axis were published in journals with higher impact if they are more variable (β = 0.61, P < 0.0001), while competition (β = -0.92, P < 0.0001) and predation (β = -0.69, P < 0.0001) studies were published in journals with higher impact when they were less variable. In addition, mutualism studies were published in journals with higher impact if they showed that species identity was important in altering the sign of the interaction outcome (β = 0.48, P < 0.0001), while competition studies were published in journals with higher impact when they showed less variation with species identity (β = -0.20, P < 0.0001).

3.17. Discussion

Variation in species interactions influences the dynamics of populations (Miller et al. 2009, Palmer et al. 2010), the structure of food webs (Travis 1996), and trait evolution (Thompson 2005). Although mean responses of participant species are often the focus of ecological studies, the variation around these means is important, but has not been given a similar level of attention (Agrawal et al. 2007,

Juliano 2009). In particular, discovering where and when we are most likely to see variation in ecological outcomes can focus research on the ecological drivers that contribute the most to variation in species interactions that influence populations, communities, and evolution.

Why mutualism is the most, and predation the least variable species interaction

Although prior research has shown that species interaction types often differ in mean interaction outcomes (Gurevitch et al. 1992, Morris et al. 2007), we know little of how species interactions differ in outcome variation. We found that that the magnitude of outcomes (CV^*_{RII}) did not differ overall among types of species interactions. However, variation in the sign of interaction outcome was the greatest for mutualism, intermediate for competition, and the least for predation, consistent with prior hypotheses that mutualisms are more variable than other types of species interactions. Greater variation in mutualism than predation is consistent with what we know about network structure. Mutualists interact with more species than predators in interaction webs (Thébault and Fontaine 2010), and specialists are more likely to have stronger, and less variable interactions (Berlow 1999, Schleuning et al. 2011). In addition, this result is somewhat consistent with differences in energy flow. Predation can minimally involve two species and the energy flow between them, leading to less variation in interaction outcomes. However, mutualism is sometimes mediated by an additional factor (e.g., ant-plant protection mediated by herbivores), providing greater potential for variation in

interaction outcomes. Although, mutualisms that are not mediated by a third party (e.g., pollination; Waser et al. 1996) are likely to be less variable. Within mutualism there are at least two more predictions about context-dependency. First, variation in outcomes should be greater in facultative mutualisms than obligate mutualisms. Species that interact with many other mutualists (e.g., free-living pollination) can sustain variable interactions outcomes, but in obligate mutualisms (e.g., yucca and yucca moths; Pellmyr 2003) large variation in outcomes may destabilize the interaction. Second, species that require a single interaction event should experience less variable interaction outcomes than those that require many. For example, many orchids require a single pollinator visit for full pollination (Petit et al. 2009), whereas other plants require many visits.

Species identity and interaction outcome variation

In addition to differences in outcome variation among species interaction types, interactions occur along many different types of gradients. Variation in interaction outcomes along gradients generates structure within populations (e.g., body size gradient; Bishop et al. 2008) creating differential fitness among individuals for natural selection to act upon (Lande and Arnold 1983), and generates structure among populations (e.g., spatial gradient; Bonser and Reader 1995). Across species interaction types, species identity drove the largest variation in magnitude and sign change of species interactions. Variation in outcome magnitude (Fig. 3) and propensity for sign change was the least for third party

presence, while abiotic, spatial and temporal contexts were intermediate. This indicates that *who* you interact with is more important than which other species are present in the community, *where* or *when* the interaction occurs, or the abiotic conditions. The importance of species identity makes intuitive sense in that focal species A interacting with species B and C will more variable than species A interacting just with species B. However, within the species identity context, the rank order of variation reversed such that predation was the most variable, mutualism the least variable, with competition intermediate (Fig. 2). This is consistent with the observation that mutualists are often generalists (pollinators: Waser et al. 1996, mycorrhizae: Smith and Read 1997) while predators are more often specialists (Gómez et al. 2010). Thus, species identity may be less important in generalized mutualisms than specialized predation interactions.

An important distinction should be made here. Species identity variation as used in this study involved a focal species interacting with *N* different other distinct species (e.g., Duncan and Chapman 2003, Reithel and Billick 2006). Third party presence studies involved the same two species interacting with or without a third species (e.g., Walls and Williams 2001, Mooney 2006). From a single species perspective species identity is the greatest source of interaction outcome variation, yet when considering two species together, varying a third party leads to very little variation in outcome. Yet, studies use the term *community context* to refer to both species identity and third party variation in interaction outcomes. Our results suggest that greater context dependency arises from species identity than

does third party, suggesting species identity is a more important component of community context than third party.

Variation among species interaction types depends on the context

Not only did variation in interaction outcomes depend on species interaction type and context gradient, but types of species interactions differed in the gradient that drove the most variation in propensity for sign change. A few patterns stand out and deserve consideration. First, mutualism had a higher propensity for sign change than both predation and competition with spatial and third party contexts. In many mutualisms, such as ant-plant protection (Heil and McKey 2003), the presence of a third party is required for benefits to accrue (Bronstein 1994). It may be this variation within a third party which causes greater variation in mutualism outcomes as variation in outcomes across locations is responsive to not just two, but three species (Fedriani et al. 2004). Second, predation had a lower propensity for sign change than both competition and mutualism with abiotic and temporal contexts. Predator-prey interactions mostly involve animals, which are mobile, and therefore may be less sensitive to abiotic conditions than plants, which make up one or both of the species in many of our competition and mutualism studies.

Variable interactions are not necessarily weak interactions

Greater variation in the outcome of a species interaction has been associated with weak mean interactions (Berlow 1999). Under this model we would expect mutualisms to be the weakest interactions because they were the most variable. However, we did not see this result (compare Figs. 1 and B1). Instead, mutualism was not different in mean outcome from predation, both of which were weaker than competition. This result bring up two points. First, mutualisms were historically considered to be less important than predation and competition because they were thought of as weak interactions (Wilson 1997). Here, we show that mutualisms are not weaker than predation and competition (Fig. B1), but mutualisms are more variable than predation and competition (Fig. 1B). Thus, it may be the variation in interactions that differentiates types of species interactions instead of their mean interaction strengths.

Variation in outcomes among locations and ecosystems

The location of ecological studies range from laboratories to greenhouses to terrestrial, freshwater, and marine systems. Location leads to differences in *mean* species interaction outcomes (Preisser et al. 2005, Hillebrand 2009), and may lead to *variation* in outcomes as well. We found that sign change was greatest in laboratory studies. This contradicts our hypothesis that variation in species interactions would be least in laboratory studies. This result may be explained by our finding that the weakest mean interaction outcomes were found in laboratory studies (Appendix C); weak mean interactions are often associated with large

variation (Berlow 1999). In addition, we found that sign change was greater in terrestrial and freshwater systems relative to marine systems, but that sign change did not differ among terrestrial and freshwater systems. Thus, interactions that occur on landmasses (including terrestrial and aquatic), whether in the water or on earth, are more variable than in the oceans. Because the oceans are larger masses of water than lakes or streams, oceans buffer abiotic change (Reid et al. 2009), which may lead to less variation in species interactions in oceans.

Methodological considerations

A common pattern in bias factors was that mutualism differed from the two antagonistic interactions. Specifically, studies on mutualism published in high impact factor journals, and published more recently, were more likely to report a change in sign, whereas studies on predation and competition published in high impact journals, and published more recently, were less likely to report a change in sign. We suggest that because the study of context-dependency has become a major theme in mutualism (Bronstein 1994, Kiers et al. 2010), researchers are deliberately investigating it in excellent studies published in top journals. In studies of other interactions, variation has not been a topic of interest and may in fact interfere with the primary objective of the study, so that a highly variable study is more likely to be published in a lower tier journal. However, even when these biases associated with the year in which the study was published and journal impact factor are accounted for, we still detect significant differences among species interaction types in sign

change. In our analysis of the effect of journal impact factor on outcome sign, impact factor was a significant predictor of sign, but species interaction type was significant ($P < 0.0001$), as well as the context gradient type ($P < 0.0001$), and their interaction ($P < 0.0001$), just as in the analysis without these bias variables (see Table 2).

Future Directions

We suggest a few steps to build on our results. First, we should compare variation in interaction outcomes for the remainder of major interaction types: herbivory, parasitism, and facilitation. This will give us a near complete picture of variation across species interactions. Both herbivory and parasitism are +/- interactions like predation, so we predict similar patterns in ecological outcome variation for herbivory and parasitism as we found for predation. Second, we should explore the gradients along which species interactions occur. Experiments manipulating multiple gradients of variation (e.g., space, time, species identity) will tease apart their relative importance, and determine if gradients of variation interact with one another. Third, phylogenetic history could be driving some patterns in our results as many ecological responses can exhibit significant phylogenetic signal (Blomberg et al. 2003). However, phylogenetic signal is more likely in ecological responses in phylogenies with young root nodes (Chamberlain et al. 2012); because a phylogeny of species in our dataset would have a very old root node given the diversity of taxa included in our study (including two of three domains: Eubacteria and Eukaryotes) our results may not change. Fourth, in this

study we combined responses at the individual and population scales. It could be that although individuals vary in their interaction outcomes, populations are buffered from fluctuations that occur among individuals. Finally, we should explore the consequences of variation in interaction outcomes. Manipulating variation in interaction outcomes empirically will be difficult, but modeling studies could explore consequences of variation in interaction outcomes for populations, communities, and evolution.

3.18. Conclusion

Variation in species interaction outcomes has been relatively unexplored compared to mean outcomes. Using meta-analysis, we showed that the major types of species interactions, predation, competition, and mutualism, differ in the extent to which they are likely to vary in sign: mutualism > competition > predation. In addition, the gradients along which species interaction occur differ in their contribution to variable interactions, with species identity associated with the most variation, and the presence of a third party species the least variation. Furthermore, types of species interactions differ in their relative amount of variance along different gradients, with predation the most variable along the species identity gradient, competition and mutualism the most variable along abiotic and temporal gradients, and mutualism the most variable along spatial and third party presence gradients. Together, these results show that species interaction types not only differ in mean interaction strengths (Appendix C; Gurevitch et al. 2000, Morris et al. 2007), but also in variance in interaction strengths.

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3.21. Tables

Table 4.1. Summary of the moderator variables used in this study.

Variables	Description	Levels of Variable
Species Interaction Type	Categorical fixed-effect variable.	-competition -predation -mutualism
Gradients of Context-Dependency	Categorical fixed-effect variable. This is the gradient along which variation in the species interaction outcome in question varies.	-abiotic (nutrients, etc. manipulated) -spatial (multiple sites) -species identity (interaction with multiple species) -temporal (data collected over time) -third party presence (third party, e.g., predator in a competition study)
Location	Categorical fixed-effect variable. Location of study.	-laboratory -greenhouse -field -outdoor tanks (aquatic or terrestrial)
Ecosystem	Categorical fixed-effect variable. Ecosystem in which study was conducted.	-freshwater (lentic or lotic) -marine -terrestrial
Paired Controls	Binary (0/1) variable. Separate control treatments are less confounded than studies without separate controls treatments.	-Separate control treatment -No separate control treatment, used same control for many experimental treatments
Publication Year	Continuous variable.	-Integer year of study
Average duration	Continuous variable.	-Length of study, in days
Impact Factor	Continuous variable.	-ISI impact factor of year study was published

Table 4.2. Summary statistics for the analyses CV* of the effect size and for the change in sign of the interaction.

Effect	*ndf,ddf*	CV^*_{RII}		Interaction Sign Change	
			P	X^2	*P*
Species interaction type	2,338	1.88	0.154	63.84	< 0.0001
Context type	4,338	7.69	< 0.0001	434.17	< 0.0001
Interaction x Context	8,338	2.88	0.004	362.47	< 0.0001

3.22. Figures

Figure 4.1 Differences among types of species interactions (p = predation, c = competition, m = mutualism) in (A) the CV*RII, and (B) the proportion of studies showing a change in the sign of the interaction (-, 0, +).

Bars show back-transformed, weighted least squares means ± 95% confidence intervals. Different letters indicate significant differences between types of species interactions ($P < 0.05$) according to post hoc Tukey HSD tests. Sample sizes for each interaction type are provided on bars in (**B**).

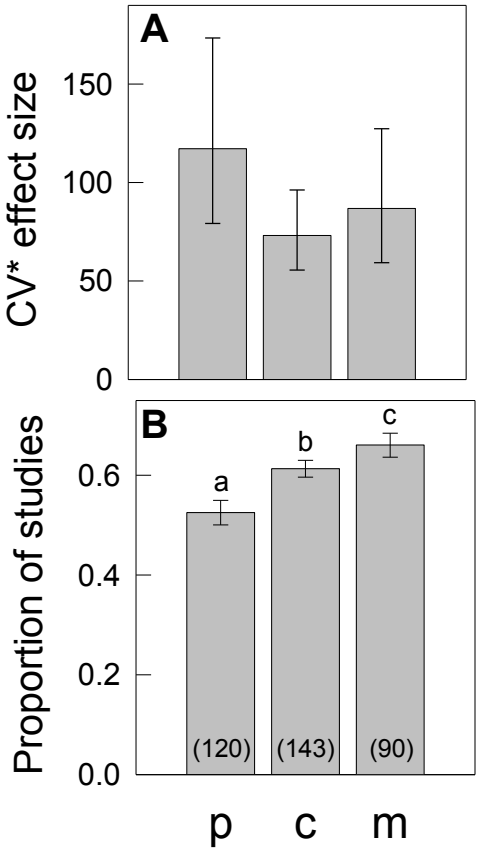


Figure 4.2 Differences in the CV* of the effect size, and the proportion of studies showing a change in the sign of the interaction among types of species interactions (p = predation, c= competition, m = mutualism) and contexts: abiotic, spatial, species identity, temporal, and third party presence.

Bars show back-transformed, weighted least squares means ± 95% confidence intervals. Within each context type, different letters indicate significant differences between types of species interactions ($P < 0.05$) according to a post hoc Tukey HSD test. Sample sizes are provided below labels on bars.

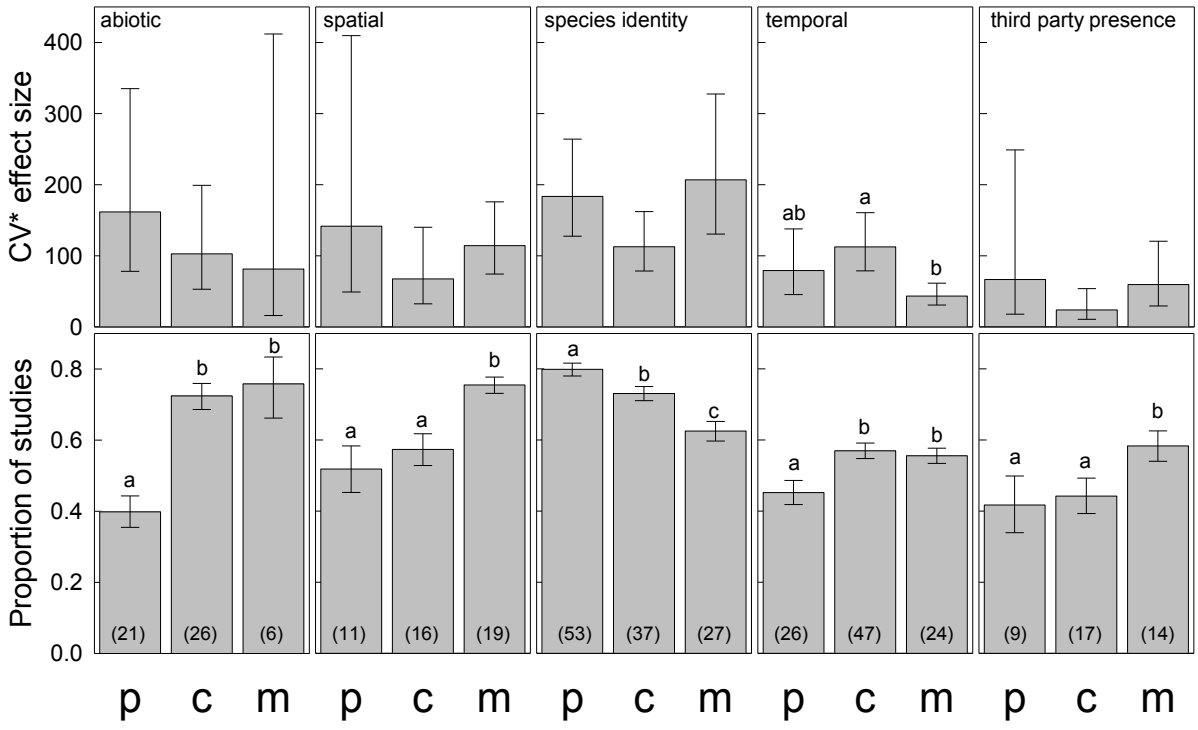
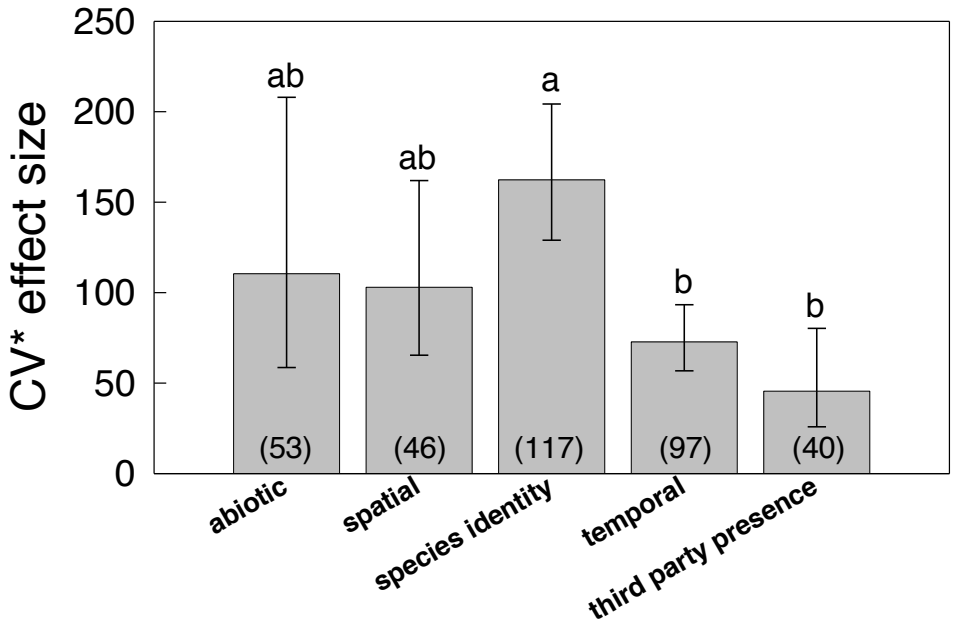


Figure 4.3 Differences in the CV*$_{RII}$ among types of contexts: abiotic, spatial, species identity, temporal, and third party presence.

Bars show back-transformed, weighted least squares means ± 95% confidence intervals. Within each context type, different letters indicate significant differences between types of species interactions ($P < 0.05$) according to a post hoc Tukey HSD test. Sample sizes for each context type are provided on bars.



3.23. Appendix A

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3.24. Appendix B

Appendix B. Mean outcome results.

Introduction

The focus of our paper was variation in species interactions. However, there are at least two reasons to examine the mean in addition to variation in the mean - one statistical and one biological. First, variation (here, CV) is calculated from one or more means, thus there is an expected relationship between variation and mean. Second, species interactions that are weaker on average are more variable (Berlow 1999).

A previous meta-analysis that focused exclusively on plants reported that interaction outcomes were weaker for positive mutualisms than for negative antagonistic interactions; however, this difference occurred only when these pair-wise interactions were isolated from other species in the community (and thus disconnected from indirect effects; Morris et al. 2007). Additional reviews have reported that effect sizes were greater for predation than competition (Sih et al. 1985, Gurevitch et al. 2000). The interaction network literature poses one possible mechanism underlying differences in effect sizes among types of species interactions. In predator-prey systems, species interact with fewer other species on average than in mutualistic systems (Thébault and Fontaine 2010). Interacting with fewer species has been linked to stronger interaction strengths in consumer-resource interactions (Edwards et al. 2010), suggesting that systems with more species

interactions per species, such as mutualisms, will have weaker average interaction strengths.

Here, we compare the mean effect sizes of species interactions among predation, competition and mutualism. We ask the following three questions: *1) Do types of species interactions (competition, predation, mutualism) differ in mean interaction outcomes*?; 2) *Does variation in magnitude of variation or propensity for sign change vary consistently with study location or ecosystem type*?; and 3) *Is magnitude of variation or propensity for sign change biased by experimental methods*?

Methods

We collected data from published studies, and calculated relative interaction intensity (*RII*) following methods in the main text. We weighted the analysis of the absolute value of the effect size using the inverse of the variance of *RII*, calculated as:

$$\frac{\frac{\sigma_W^2}{n}+\frac{\sigma_O^2}{n}}{(B_W+B_O)^2}\left(1+\frac{(B_W-B_O)^2}{(B_W+B_O)^2}-\frac{2*p*(B_W-B_O)}{B_W+B_O}\right).$$

We pooled variance when taking the mean across context levels within a study. Data analyses were conducted following methods used in the main text for CV^*_{RII} and sign-change.

Results

1) Do types of species interactions (competition, predation, mutualism) differ in mean interaction outcomes?

Absolute effect sizes (|*RII*|) spanned nearly the full range of possibilities (range: 0.001 – 0.98). The effect sizes of positive interactions were neither weaker than predation nor stronger than competition (Fig. 1). On average, studies on competition had 88% higher mean effect sizes than studies on predation, whie studies on mutualism were not different in mean effect size from predation or competition ($P = 0.048$; Table 1). In addition, mean effect size differed across context axes ($P < 0.0001$); on average, interactions were 237% greater across abiotic than species identity axes, were 194% greater across spatial relative to species identity, and 174% greater across temporal relative to species identity. Differences among species interactions marginally depended on the context axis (Fig. 2, interaction × context, $P = 0.051$, Table 1). Predation studies were weaker than competition and mutualism studies when studies examined species identity and temporal variation (Fig. 2). Competition and mutualism studies did not differ in mean effect sizes within species identity and temporal contexts. Caution is advised when interpreting the lack of differences in some contexts between species interaction types as the sample size for mutualism studies that examined interactions across abiotic contexts was small ($n = 6$), and sample size for predation studies that examined interactions with third party presence was small as well ($n = 9$).

2) *Does variation in magnitude of variation or propensity for sign change vary consistently with study location or ecosystem type*?

Location of the study had a significant influence on the mean effect size ($F_{8,364}$ = 6.21, P = 0.0004). The location effect was driven by the difference between two of the four study locations (laboratory, greenhouse, outdoor tanks, field): there was a 78% higher mean effect size in studies conducted in outdoor tanks (terrestrial or aquatic) than in field studies not in enclosed containers (Tukey post-hoc test, $P < 0.05$). Although mean effect sizes for marine ecosystems were 14% larger than freshwater ecosystems (lentic + lotic), and were 47% larger than terrestrial ecosystems, the difference in mean effect sizes among ecosystems was not significant ($F_{2,348}$ = 1.63, P = 0.198).

3) *Is magnitude of variation or propensity for sign change biased by experimental methods*?

Studies that used a separate control for each context in the experiment had effect sizes that were 626% larger than effect sizes in those studies without separate controls ($F_{1,351}$ = 25.99, $P < 0.0001$). However, average duration of the study (β = 0.03, P = 0.671) had no overall influence on mean effect size. We expected that studies published in journals with higher impact factors would have larger effect sizes. Indeed, this was the case: studies with larger journal impact factors had larger mean effect sizes (β = 0.31, $P < 0.0001$). Furthermore, studies published more recently have larger mean effect sizes (β = 0.07, $P < 0.0001$).

Discussion

We quantitatively examined the magnitude of species interactions. With a meta-analysis of 395 studies, we showed that competition has the highest mean interaction outcome, followed by predation and mutualism, which did not differ from each other. In a meta-analysis Gurevitch et al. (2000) found that competitors had larger effects on organisms' growth than did predation, but predation had larger effects on organisms' survival than did competition. In our study, we pooled results across response variables, suggesting that across organisms' responses, competition is a stronger species interaction than predation. Our results are inconsistent with the findings of Sih et al. (1985) who found that predation had larger effect sizes than competition. However, we argue that our results are the most robust since our database is the most comprehensive and includes only experimental manipulations of species interactions. In addition, it is important to keep in mind that the relative strength of predation, competition, and mutualism are likely to vary depending on the response variable (e.g., growth versus survival).

Despite these overall patterns, differences in the magnitude of species interactions varied strongly with the axis along which the interaction occurred. Across species interaction types, mean interaction effects were weakest when interacting with different species. These weak mean interactions across species identity are associated with the most variable interactions (see main text). This result is consistent with Berlow (1999), who showed that weak predator-prey

interactions are the most variable. Last, consistent with the findings of Menge et al. (1994), in this paper mean interaction strength for predation was 146% greater in spatial relative to temporal contexts (Fig. 2), although the difference was not significant in their paper or this one.

Many bias factors influenced our analyses. First, how the study is conducted is important. We found that studies that used separate controls had much smaller effect sizes, suggesting that studies that don't use separate controls are overestimating effect sizes. However, study duration had no influence on mean effect sizes. This result contrasts with the meta-analysis of Borer et al. (2005), who found that effect sizes of trophic cascades increased with study duration, but is consistent with some studies that have found no relationship (e.g., Harsch et al. 2009, Barto and Rillig 2010). Second, where the study is conducted is relatively important. That is, studies conducted in enclosed containers (i.e., tanks) overestimate effects relative to field studies not in enclosed containers. Third, the publishing context is important. Studies published more recently in journals with higher impact factors had larger effects. This makes sense because less variable effect sizes result in smaller sampling errors around mean effects – which are likely to be viewed more favorably in higher impact journals.

Our results show that mean species interactions vary more among context axes than among types of species interactions. This suggests the novel finding that regardless of the type of species interaction, interaction magnitude will be highly dependent on the axis along which the interaction occurs. Specifically, interactions

are relatively weak when interacting with different species. Further research should further explore the mechanisms behind mean interaction outcome variation along axes of context-dependency, and explore how the axes interact to influence species interactions.

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Table B1. Summary statistics for the analyses of absolute mean effect size of interaction outcomes.

		Mean |*RII*|	
Effect	*ndf,ddf*	*F*	*P*
Species interaction type	2,338	3.06	0.048
Context type	4,338	12.22	< 0.0001
Interaction x Context	8,338	1.96	0.051

Figure B1. Differences among types of species interactions (p = predation, c = competition, m = mutualism) in the absolute value of the effect size (|*RII*|). Bars show back-transformed, weighted least squares means ± 95% confidence intervals. Different letters indicate significant differences between types of species interactions ($P < 0.05$) according to post hoc Tukey HSD tests. Sample sizes for each interaction type are provided on the bars.

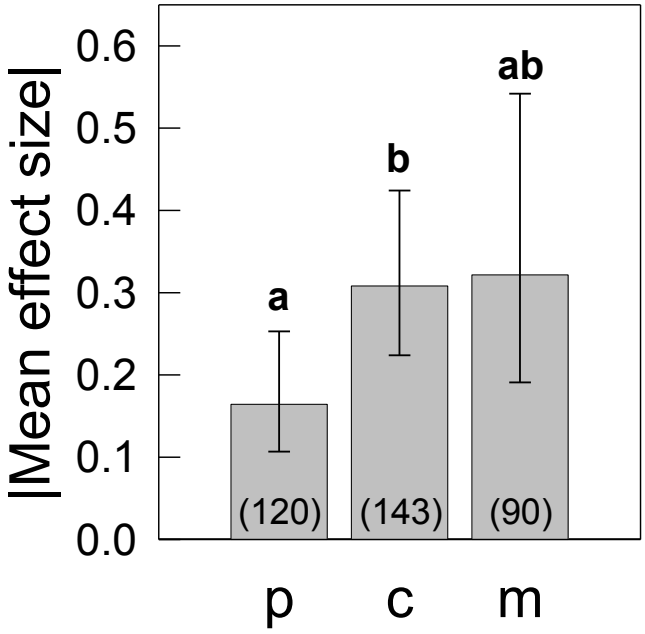

Figure B2. . Differences in the absolute effect size (*RII*) among types of species interactions (p = predation, c= competition, m = mutualism) and contexts: abiotic, spatial, species identity, temporal, and third party presence. Bars show back-transformed, weighted least squares means ± 95% confidence intervals. Within each context type, different letters indicate significant differences between types of species interactions ($P < 0.05$) according to a post hoc Tukey HSD test. Sample sizes are provided below labels on the bars.